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GRAY LEAF SPOT OF RAPE IN MANITOBA[1]

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ABSTRACT

A disease of rape characterized by black spots on stems and pods and gray to black spots on the leaves, caused considerable damage to crops in Manitoba in the wet years 1955 and 1956. *Alternaria brassicae* (Berk.) Sacc. was the only pathogen isolated from infected leaves, stems, pods and seed. Sporulation of the fungus was greatest on a 10% alfalfa seed decoction agar medium. The fungus was pathogenic to seedlings and adult plants. Penetration into leaves was indirect through stomata. Inoculations in greenhouse and field experiments resulted in a higher percentage of shrunken seed and seed infestation by the fungus, and in lower yield and percentage germination of seed compared with those from uninoculated plants.

INTRODUCTION

The acreage sown to oilseed rape (*Brassica napus* L.) in Western Canada has increased sixfold in the past 3 years. In Manitoba there were 29,200 acres of this crop in 1958 compared to 5,200 acres in 1955. This sharp increase in production has resulted in the appearance of diseases which already are important or may become important in the culture of this crop. One of these diseases, known as gray leaf spot (11), caused by the fungus *Alternaria brassicae* (Berk.) Sacc., occurred in epidemic form in several years and is likely to become a limiting factor in years favourable to its development.

Two species of *Alternaria* cause very similar symptoms on cruciferous seed crops and, as both may occur on the same plant, it is often difficult to assess their relative importance in any one area. The second species is *A. brassicicola* (Schw.) Wilts. and the disease it causes has been named black leaf spot (11) to distinguish it from that caused by *A. brassicae*. In Europe other descriptive names such as speckle disease and dark leaf and pod spot (9) are used for the disease in general as both species may be present and the symptoms may be indistinguishable. The taxonomy of the two species was so confused prior to 1947 (6, 12) that in many cases it was impossible to determine to which species the disease was being attributed in reports in the literature. Since then several papers on the disease on rape have appeared from Europe but no general agreement has been reached as to which organism is primarily responsible (1, 2, 9). Groves

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and Skolko (3) isolated only *A. brassicae* from rapeseed in Canada but on seed of other crucifers found that *A. brassicicola* was more prevalent and more pathogenic.

The disease was first reported on rape in Manitoba in 1955 (8) causing severe damage in the interlake area. In 1956 it appeared in epidemic proportions in most parts of Manitoba but in 1957 and 1958 only slight damage was reported late in the season. It has been recognized in Saskatchewan since 1948 (10). Because of the possible importance of the disease in Western Canada and the apparent uncertainty as to the relative importance of the two species a study was made to determine: 1) the pathogen involved in the disease on rape in Manitoba; 2) methods of screening varieties and selections for resistance to the disease; 3) losses caused by the disease in the field.

SYMPTOMS

The most conspicuous field symptoms of the disease caused by *A. brassicae* are the black spots which occur on the stems and pods (Fig. 4). These begin as small brown to black points which change in colour as they enlarge, becoming either entirely black or dark bordered with a grayish-white centre. The leaf spots vary in size and colour depending on environmental conditions and may be entirely gray under moist conditions (Figure 1), gray with a black border, or entirely black under less favourable conditions (Figure 2). The tissue surrounding the spots on leaves becomes chlorotic and defoliation results. Severely spotted pods become dry and shrunken and split prematurely with a consequent loss in yield. Those spotted pods that are harvested contain shrunken seed infested with the fungus. Such infested seed gives rise to diseased seedlings which may be killed either before or after emergence. The hypocotyls of surviving seedlings may be infected, causing a wire stem symptom, and the cotyledons may be spotted. Spores produced on these infection sites may spread to healthy plants.

DISTRIBUTION IN MANITOBA

A. brassicae was the only pathogen isolated from spotted leaves, stems, pods, and seed of rape collected in Manitoba in 1956. It was too late in the year for a disease survey to be made to determine the distribution of the disease and in lieu of this the amount of seed infestation in 25 samples of registered seed grown in different parts of the province was determined. Two lots of 100 seeds from each sample were examined for seed-borne fungi. One lot was plated without treatment on filter paper over wet vermiculite in petri dishes and the seeds were examined after 10 days. The other lot was surface sterilized by immersion for one-half minute in 50 per cent alcohol followed by immersion for 1 minute in 2 per cent sodium hypochlorite solution, and was then plated on potato sucrose agar (PSA). Four days after plating, fungus colonies on the agar plates were examined and those of *A. brassicae* counted. Isolates of other colonies proved to be variants of *A. tenuis* auct. sensu Wiltshire which was the most prevalent species encountered on seeds plated by either method. Seeds infested with

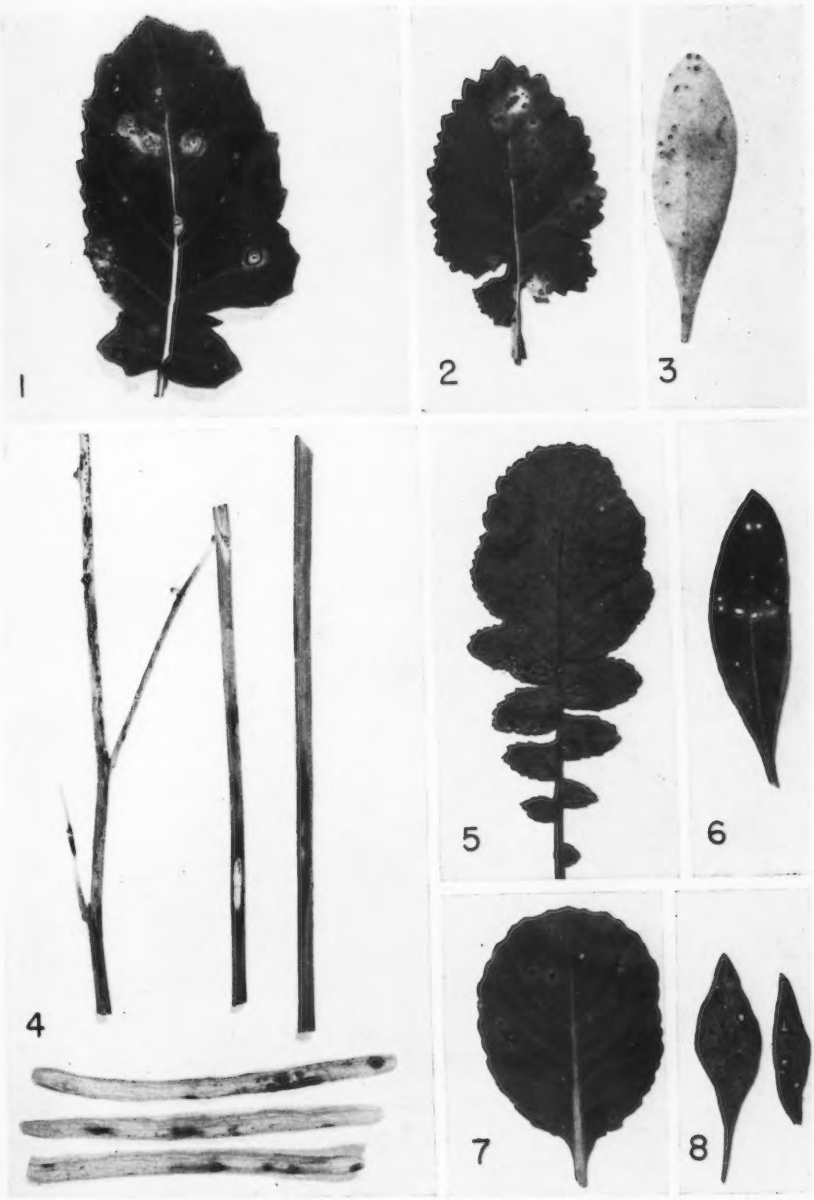

FIGURE 1. Gray leaf spot symptoms on rape leaf in humid atmosphere.
FIGURE 2. Gray leaf spot symptoms on rape leaf in dry atmosphere.
FIGURE 3. Gray leaf spot symptoms on leaf of stocks.
FIGURE 4. Symptoms of artificial infection with *A. brassicae* on stems and pods of rape.
FIGURE 5. Gray leaf spot symptoms on leaf of radish.
FIGURE 6. Gray leaf spot symptoms on leaf of wallflower.
FIGURE 7. Gray leaf spot symptoms on leaf of cabbage.
FIGURE 8. Gray leaf spot symptoms on leaf of candytuft.

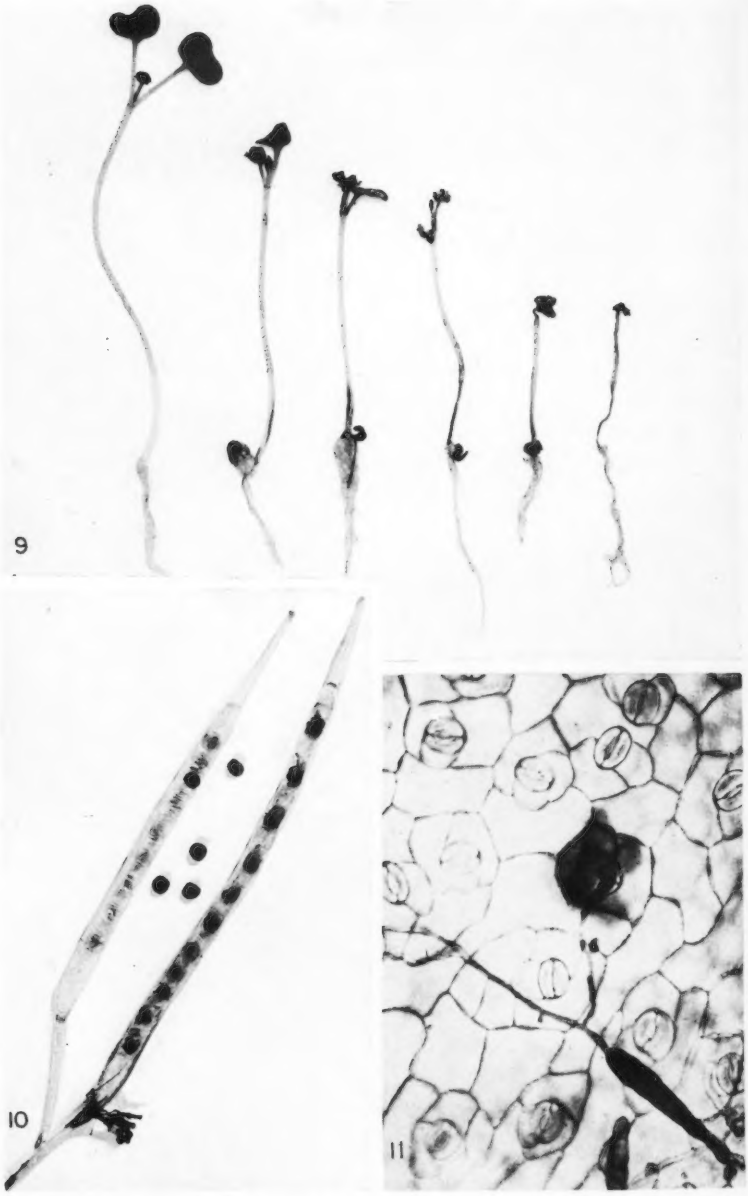

FIGURE 9. Symptoms of blight on rape seedlings grown in sand infested with *A. brassicae.* Uninoculated check at left.

FIGURE 10. Growth of *A. brassicae* mycelium inside inoculated rape pod. Uninoculated check pod at left.

FIGURE 11. Penetration into stoma of rape leaf by germ tube from a spore of *A. brassicae.*

A. brassicae were found in 14 of the 25 samples. The highest percentage obtained was 4 per cent. This probably did not indicate the true percentage in the crops in those areas because of the careful cleaning of registered seed samples which removes most of the shrunken seeds. The results did indicate that the disease was widespread in Manitoba in 1956.

CULTURAL CHARACTERISTICS OF A. BRASSICAE

The morphological characteristics of the pathogenic *Alternaria* isolated from rapeseed in Manitoba agreed with those of *A. brassicae* published by Neergaard (6). Spores measured 129 (80 – 177) x 23 (17 – 27) μ, were prominently beaked when fully developed, and did not form chains. Colonies on PSA and V-8 juice agar consisted of pinkish-white mycelium with few conidia. More conidia were produced on V-8 juice agar, usually in concentric rings in the central portion of the colony. Many authors (3, 6, 7) have observed that the fungus sporulates poorly on agar media in contrast to natural media. It sporulates abundantly on plated, naturally infested rapeseed. With the object of obtaining a medium on which copious spore production could be induced, autoclaved seeds of rape, alfalfa, beans, sorghum, and barley in medicine bottles were inoculated with a spore suspension of the fungus and incubated for 2 weeks. Only alfalfa seed was found to stimulate sporulation. A 10 per cent alfalfa seed decoction agar (ADA) was then compared with V-8 and PSA as a medium for sporulation. Single spores from each of 3 isolates of *A. brassicae* were transferred to duplicate tubes of each medium and incubated at room temperature for 17 days. Spore suspensions were made from each tube by adding 10 ml. of water and gently rubbing the surface of the colony with a wire loop. Each suspension was strained through a layer of fine cheesecloth and the spore concentration was determined by making duplicate counts with a haemycytometer. The results are given in Table 1.

The colonies on ADA were covered with typically large, olive-brown conidia and, despite about a one-third reduction in the size of the colony, yielded 5 times as many conidia as those grown on V-8 agar. This copious production of conidia on ADA occurred only if the tubes were seeded with a single spore. Tubes or plates of any one of the three media flooded with a conidial suspension yielded fewer spores and these were

TABLE 1.—SPORE PRODUCTION BY SINGLE-SPORE ISOLATES OF *A. brassicae* ON VARIOUS MEDIA

Isolate Number	Potato sucrose agar		V-8 agar		Alfalfa decoction agar	
	Colony diam. (mm.)	No. of spores/cc.	Colony diam. (mm.)	No. of spores/cc.	Colony diam. (mm.)	No. of spores/cc.
1	80+	2300[1]	80+	5100	30	28300
2	80+	2100	80+	7400	20	26800
3	80+	4100	80+	5700	32	45900
Average	80+	2833	80+	6067	27	33667

[1]Average of 2 counts from each of 2 tubes.

small and atypical. When several single spores were transferred separately to a plate of ADA conidial type colonies developed which were mutually antagonistic. This did not happen on V-8 juice agar where no inhibition between single spore colonies was observed.

Van Schreven's results (9) indicate good sporulation on cherry juice agar. This was verified but, as cherry juice is not readily obtained locally, alfalfa seed decoction has been used exclusively in this work. Colonies obtained by successive single spore transfers on ADA have not changed morphologically or pathogenically in the past 2 years.

INFECTION STUDIES

Pathogenicity

A method described by Halpin *et al.*(5), slightly modified, was used to test the pathogenicity of Manitoba isolates of *A. brassicae* to rape seedlings, and the effect of temperature on disease development. Twenty surface-sterilized seeds of Argentine rape were transferred to each of 32 jelly jars containing sterile sand. One ml. of spore suspension was aseptically pipetted over the seed in each of 16 jars and the seed in all jars was covered by 15 cc. of sterile sand. Four jars of inoculated seed and four of uninoculated seed were placed in growth cabinets at temperatures of 55°, 70°, 75°, and 80°F. respectively. Fourteen days later the seedlings were removed from the jars and rated for disease.

The greatest amount of disease developed at a temperature of 70°F. The symptoms on emerged seedlings (Figure 9) consisted of a rotting of the cotyledons and necrosis of the hypocotyls similar to the wire stem reported by Rangel (7). In some cases the germinated seedling rotted before emergence. These symptoms are similar to those reported on many cruciferous hosts attacked by different species of *Alternaria*.

Symptoms of the disease as observed in the field were reproduced on maturing rape plants in the greenhouse by inoculation with a spore suspension of the fungus. Eleven plants of the variety Golden, all with young pods, were inoculated by atomizing them with a spore suspension of approximately 150,000 spores per cc. supplemented with gelatin to make a 0.25

TABLE 2.—EFFECT OF INOCULATION WITH *A. brassicae* ON SIZE, GERMINATION AND INFESTATION OF RAPESEED

Class	Seed size				Germination		Infestation	
	Check number	%	Inoculated number	%	Check %	Inoculated %	Check %	Inoculated %
Large	1146	66	148	16	97	94	1	12
Medium	545	32	701	77	94	57	0	49
Small	33	2	61	7	55	11	0	67
Total	1724		910					

TABLE 3.—EFFECT OF INFECTION WITH *A. brassicae* ON YIELD, SEED SHRINKAGE, AND SEED INFESTATION OF RAPESEED, VARIETY ARGENTINE

Treatment number	Time of inoculation	Pod spotting	Mean yield (gm.)	Shrunken seed (%)	Infestation (%)
8	Not inoculated. 2 Captan sprays	trace	549	2.6	0.8
7	Not inoculated	trace	521	3.2	0.8
6	Young pods (YP)	moderate	519	6.7	11.2
1	Pre-bloom (PB)	trace	504	3.7	1.8
3	Flowering (FL)	light	479	4.4	4.8
2	PB and FL	light	468	4.3	5.6
5	FL and YP	moderate	461	6.9	10.0
4	PB, FL and YP	moderate	440	6.7	16.8
L.S.D. (P = .05)			N.S.	N.S.[1]	1.7[1]

[1] Angular transformation applied before analysis

per cent solution. Five other plants were sprayed with a 0.25 per cent gelatin solution and used as checks. All plants were placed for 3 days in a plastic humidity chamber at 70°F. and sprayed intermittently to maintain a high humidity, after which they were returned to a greenhouse bench at the same temperature. The symptoms produced are illustrated in Figure 4.

Seed from spotted and clean pods harvested 6 weeks after inoculation was compared to determine any differences in size, germination and infestation by *A. brassicae*. This seed was screened through appropriate sieves to yield "large", "medium", and "shrunken" seed classes. One hundred seeds, where possible, from each size class from the clean and spotted pods were plated directly on filter paper and examined 6 days later. The results are given in Table 2.

Although the experiment was on a small scale the results show that reduction both in the size of the seed and percentage germination was correlated with seed infestation with *A. brassicae*.

A more extensive experiment was designed in 1958 to determine the feasibility of producing infection in the field, and to obtain an estimate of actual losses caused by the disease. A randomized block design was used containing 40 plots of 4 rod rows each of Argentine rape with a guard row of sweet corn around each individual plot and a 1-foot path between each 2 plots. Eight treatments were used, each replicated 5 times. Six of the treatments consisted of inoculations at one or more stages of growth as indicated in Table 3, and the other two were uninoculated checks. Treatment No. 8 was sprayed twice with Orthocide 50W (50 per cent Captan) at 4 pounds per acre at the pre-bloom stage and at flowering, to prevent secondary infection from wind-blown spores. Inoculum was prepared from 11-day-old cultures of the fungus grown on 100 ml. of V-8

juice liquid medium in 32-ounce medicine bottles at 72°F. Each bottle had been inoculated with 10 ml. of a spore suspension obtained from one 14-day-old colony grown on ADA. It was determined, through measurements of mycelial growth in bottles under these conditions over a period of 26 days, that the fungus colony reached its maximum weight at 11 days. The mycelial mats from the medicine bottles were macerated with tap-water in a Waring blendor and gelatin was added to make a final solution of 0.25 per cent. Thirty medicine bottles provided enough inoculum to make 12 litres of suspension, sufficient to inoculate 20 plots. The plots were sprayed in the evening. The centre 2 rows of each plot were harvested and the yield, percentage of shrunken seed, and percentage of seed infestation were determined. The shrunken seed was separated with an air-blast seed separator set to remove seeds weighing between 1.5 and 2 grams. per 1000 seeds. Seed infestation was determined by plating 100 shrunken seeds from each plot. The results are illustrated in Table 3.

Hot, dry weather prevailed during most of the summer and as a result very little natural spread occurred between plots. Satisfactory infection was produced by each inoculation despite the unfavourable weather. Spraying both before and after flowering resulted in considerable defoliation through leaf spotting and numerous spots on the young stems. Spraying of young pods produced abundant spotting of most pods; some escaped infection because of lodging before inoculation. Many infected pods split open before harvest. The differences in yields and percentage of shrunken seed between treatments were not statistically significant, although very strong trends were evident. The differences in seed infestation between treatments were significant, the highest percentage occurring in inoculated pods, as would be expected. This test showed that it is possible to obtain good infection by field inoculations even under adverse weather conditions. Difficulties in accurate harvesting and differences in emergence between replicates because of spring flooding contributed towards the high experimental error. If these difficulties can be overcome, significant differences should be obtainable with the same series of inoculations that was used in 1958.

Host Range

A. brassicae attacks most species in the genus *Brassica* as well as plants in other genera such as *Raphanus sativus* L. (radish), *Armoracia lapathifolia* Gilib. (horseradish), *Cheiranthus cheiri* L. (wallflower), *Iberis amara* L. (candytuft), *Matthiola incana* (L.) R. Br. (stocks), *Lepidium sativum* L. (garden cress), and others (6). The susceptibility of the following hosts was verified by inoculations in the greenhouse: *R. sativus*, *C. cheiri*, *I. amara*, *M. incana*, *B. oleracea* (cabbage, cauliflower), *B. napus* (rape, varieties Dwarf Essex, Argentine, Golden, and Polish) and *L. latifolium* L. (broad-leaved cress). Characteristic symptoms are illustrated in Figures 2, 3, 5, 6, 7 and 8.

Penetration

Domsch (2) showed that germinating spores of *A. brassicicola* usually penetrated directly through the epidermal cell wall. The method of penetration by spores of *A. brassicae* was determined by examining sections

of inoculated leaves of radish and rape plants. These sections were decolorized 24 hours after inoculation by boiling in a 1:1 solution of 95 per cent alcohol and acetic acid. In all cases the germ tubes had penetrated the leaves through stomata as illustrated in Figure 11. The palisade cells in the area beneath the point of infection were discolored.

Environmental Factors Affecting Disease

Environmental factors may have some bearing on the relative importance of the two *Alternaria* species which attack rape in any one area. The optimum temperature for disease development was shown to be about 70°F. (21° C.) when seedlings and detached leaves inoculated with *A. brassicae* were incubated at various temperatures. Van Schreven (9) reported optimum development of disease at temperatures between 20° and 24°C. (68° and 75°F.). The optimum temperature for mycelial growth was reported to be 23°C. (73°F.) (6) and for spore germination between 17.2° and 21.1°C. (63° and 70°F.) (7). The corresponding temperatures for *A. brassicicola* are higher; 24°C. (75°F.) for disease development, 25°C. (77°F.) for mycelial growth, and 30°C. (86°F.) for spore germination (2). Most authors (2, 7, 9) agree that protracted rainy periods are necessary for good infection by both fungi. Van Schreven (9) notes that conidia of *A. brassicae* germinate more readily in humid air than conidia of *A. brassicicola*. Domsch (2) states that for an epidemic of *A. brassicicola* to develop a relative humidity of 95 to 100 per cent must be maintained for at least 18 hours with a temperature between 21° and 27°C. (70° and 80°F.) for at least 3 successive days.

These data indicate that only rarely would conditions in Manitoba favour the development of an epidemic of *A. brassicicola*. In 1956, although rainfall was double the normal, there were only 4 scattered days in August when the temperature reached a mean of 70°F. (21°C.) or over. However, as *A. brassicae* can develop at lower temperatures than *A. brassicicola*, the conditions were sufficiently near optimum for an epidemic of the disease caused by *A. brassicae* to develop.

Premature Germination

When plants bearing pods were inoculated with *A. brassicae* and grown to maturity, it was found that in some cases the mycelium had penetrated inside the pod, completely enveloping the seed. The seed coat had rotted in part and some of the seeds had germinated *in situ* (Figure 10). When inoculated and check pods were placed in petri dish moist chambers, the inoculated pods became covered with spores of *A. brassicae* and the interior was overgrown with mycelium. The check pods were covered with contaminants but no mycelium was formed inside. Grümmer (4) reported that both the spores of *A. brassicae* var. *somniferi* Br. & Har. (probably a strain of *A. tenuis* according to Neergaard (6)), and the filtrate from its culture, when injected into poppy (*Papaver somniferum* L.) capsules containing half-ripe seed, induced premature germination within the capsule. Injections of sterile water or sterile Czapek's medium had no effect. The possibility of a similar stimulation of rapeseed germination through infection by *A. brassicae* is being investigated.

DISCUSSION

Investigations on the dark leaf and pod spot disease of rape in Europe emphasize its importance as a factor in seed production. They also emphasize the lack of agreement on the relative importance of the two causal organisms. Domsch (2) in Germany reports losses of rapeseed of up to 75 per cent of the crop and in his studies on the disease found most of the damage attributable to *A. brassicicola*. Neergaard (6) states that *A. brassicicola* is the more prevalent in isolations from crucifer seeds in Denmark and is more pathogenic in seedling tests. Van Schreven (9) in Holland estimated a loss of 1070 pounds per acre (1200 kg. per hectare) of turnip rapeseed in one area in 1949 and in 1950 a loss on 4446 acres (1800 hectares) of winter rapeseed of 250,000 dollars (1 million guilders). He isolated both fungi from seed and spots on pods and stems but *A. brassicae* predominated. In pathogenicity tests on seedlings he found *A. brassicicola* to be more pathogenic but on older plants inoculations with *A. brassicae* induced more typical field symptoms. Darpoux *et al* (1) in France believe that *A. brassicae* is the more important of the two on rape and radish.

Whether *A. brassicae* or *A. brassicicola* causes the black spot disease on rape in any one area is important from the standpoint of control both through the development of resistant varieties and the use of fungicides. The isolations made from infected material and the weather conditions occurring in this area leave little doubt that *A. brassicae* has been responsible for the losses caused to rapeseed crops through pod spotting in Manitoba and Saskatchewan. Therefore any efforts made to control the disease should be directed against this species.

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NUTRITIONAL QUALITY OF FORAGE CROPS ADAPTED TO SOUTHWESTERN SASKATCHEWAN AS DETERMINED BY THEIR DIGESTIBILITY AND DRY MATTER INTAKE WHEN FED TO SHEEP[1]

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ABSTRACT

Hays from stands top-dressed with 150 pounds of ammonium nitrate (33.5 per cent nitrogen) per acre and unfertilized stands of crested wheatgrass, *Agropyron cristatum* (L.) Gaertn., intermediate wheatgrass, *A. intermedium* (Host.) Beauv., streambank wheatgrass, *A. riparium* Scribn. and Smith, and Russian wild ryegrass, *Elymus junceus* Fisch., as well as hay from unfertilized stands of tall wheatgrass, *A. elongatum* (Host.) B. P., reed canary grass, *Phalaris arundinacea* L., and Ladak alfalfa, *Medicago media* Pers., were fed to 70-pound wethers in digestion stalls in a randomized incomplete-block experiment.

Chemical analyses of the hays indicated differences in percentage organic matter, crude protein, and nitrogen-free extract, while feed-feces ratios showed differences in the percentage digestibility of crude protein, nitrogen-free extract, and crude fibre. Despite these results no differences were established in nutritive values as estimated by digestible dry matter, total digestible nutrients, digestible energy or starch equivalents.

Calculations demonstrated that the nutrient intake of crested wheatgrass, streambank wheatgrass, Russian wild ryegrass, and Ladak alfalfa were sufficient to provide at least a maintenance ration, but that the voluntary nutrient intake of intermediate wheatgrass, tall wheatgrass, and reed canary grass did not do so.

Top-dressing did not increase the digestibility nor the apparent palatability of any feed; apparently consumption was determined by species characteristics rather than fertilizer treatment.

LITERATURE REVIEW AND INTRODUCTION

During the past three decades several previously little known perennial forage plants have been plot- and field-tested throughout southern Saskatchewan to measure their tolerance to the semi-arid continental climate and their response on the various soil types of the region (10, 22). Determinations of their proximate principles, and mineral and lignin contents, have been undertaken to estimate nutritive qualities (4, 5, 11, 20). Recommended cultural practices (22), and ecological relationships (9, 19), have been reported.

Crested wheatgrass, *Agropyron cristatum* (L.) Gaertn., was recognized quickly as a species adapted to the climate and to a variety of soil types; its usefulness for both spring pasture and hay was demonstrated by experimentation (23) and farm experience. Likewise, Ladak alfalfa, *Medicago media* Pers., was accepted by stockmen when experimentation demonstrated that it had somewhat greater drought, disease, and cold tolerance than other alfalfa varieties (7). Reed canary grass, *Phalaris arundinacea* L., has been used extensively to regrass spring flooded meadows (29). More recently the proven drought tolerance and higher autumn crude protein content

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TABLE 1.—GENERAL DESCRIPTION OF FEEDS

Crop	Feed No.	Description
Crested wheatgrass *Agropyron cristatum* (L.) Gaertn.	1.	Unfertilized. Late bloom stage. About 2.5′ tall.
	2.	Top-dressed with 150 lb. of ammonium nitrate (33.5% nitrogen) per acre in October of each year. More mature than No. 1, 3′ tall.
Intermediate wheatgrass *A. intermedium* (Host.) Beauv.	3.	Unfertilized. Early seed stage. Very leafy, 3′ tall.
	4.	Top-dressed with 150 lb. of ammonium nitrate (33.5% nitrogen) per acre in October of each year. Leafy and taller than No. 3.
Streambank wheatgrass *A. riparium* Scribn. and Smith.	5.	Unfertilized. Very leafy, few stems, about 1½′ tall.
	6.	Top-dressed with 150 lb. of ammonium nitrate (33.5% nitrogen) per acre in October of each year. Slightly more mature than No. 5.
Russian wild ryegrass *Elymus junceus* Fisch.	7.	Unfertilized. Basal leaves only, about 10″ tall.
	8.	Top-dressed with 150 lb. of ammonium nitrate (33.5% nitrogen) per acre in October of each year. A few heads in dough stage, very leafy.
Reed canary grass *Phalaris arundinacea* L.	9.	Unfertilized but spring flooded, stemmy, 4′ tall, leafy and green, early flowering stage.
Tall wheatgrass *A. elongatum* (Host.) B.P.	10.	Unfertilized and sub-irrigated, stemmy, 4.5′ tall, leafy and green, early flowering stage.
Ladak alfalfa *Medicago media* Pers.	11.	Unfertilized. Mid-flowering, stemmy but still green and leafy.

of Russian wild ryegrass, *Elymus junceus* Fisch., have encouraged its use (21). Three other grasses have valuable properties: intermediate wheatgrass, *A. intermedium* (Host.) Beauv., establishes stands quickly and produces relatively high hay yields; streambank wheatgrass, *A. riparium* Scribn. and Smith, is drought-tolerant and develops a dense turf; tall wheatgrass, *A. elongatum* (Host.) B. P., shows considerable tolerance to salinity and high water tables (9, 22).

The study being reported in this paper was undertaken to assess the dry matter digestibility and nutritive qualities of the seven species noted above when harvested for hay and fed to lambs.

MATERIALS AND METHODS

Blocks of crested wheatgrass, intermediate wheatgrass, streambank wheatgrass, and Russian wild ryegrass had been seeded in 1950 and included in a fertilizer experiment from 1952 through 1955. Hay from plots top-dressed with 150 pounds of ammonium nitrate (33.5 per cent nitrogen) per acre in October of each year, and from unfertilized plots, was harvested during early July, 1955, for inclusion in the feeding trial; nearby stands of unfertilized Ladak alfalfa, reed canary grass, and tall wheatgrass were harvested at the same time for inclusion in the same test. All crops would have been classed as very good winter roughage—relatively clean, sweet-scented, and green in colour. All were field cured, baled, and stored outside until needed. Prior to the commencement of the feeding test, all feeds were chopped into 1- to 1½-inch lengths by a forage cutter. General descriptions of the 11 feeds are presented in Table 1.

Five wether lambs of Rambouillet breeding were confined in digestion crates designed for separate collection of feces and urine. During pre-collection periods they were exercised in a small corral each afternoon. They were weighed daily prior to the morning feeding and watering. Half of each day's ration was fed at 9.00 a.m. and again at 4.00 p.m. A cobaltized salt block was placed in the feeding compartment of each crate.

The 11 feeds were fed to the 5 lambs in a randomized balanced incomplete-block experiment, with 11 treatments (feeds), 11 blocks (collection periods) with 5 observations per block (lambs). This design allowed each feed to be fed with all others during two periods. The feeding plan was that proposed by Cochran and Cox—# 11.19, page 390 (12).

Each feeding period continued for 21 days, and comprised a pre-collection period of 11 days followed by the collection period of 10 days. During the pre-collection period each lamb's appetite for a particular feed was determined by increasing or decreasing the daily ration. The determined daily rations were then weighed into paper bags, one for each of the two daily feedings. Samples were removed for chemical analyses wh n the daily rations were being parcelled. Refused portions of any feed were removed and returned to their respective bags prior to the next feeding; at the end of the collection period these were bulked, dried, weighed and prepared for chemical analyses.

Feces were collected daily. Each day's collection was added to an assigned container and kept frozen until that particular collection period ended. The bulked feces were then dried in a forced air-draft oven at 60° C. for 48 hours, and the dry weight recorded as feces dry matter.

The hay, refused feed, and feces were analysed for proximate principles, lignin, and gross energy. Proximate principles were determined as recommended by A.O.A.C. (2); lignin contents were estimated according to the procedure of Ellis *et al.* (14); a Parr peroxide bomb calorimeter was employed to estimate gross energy. In the few cases where chemical analyses

TABLE 2.—CHEMICAL COMPOSITION OF FORAGE (Per cent of Dry Matter)
(Mean of 5 duplicate determinations)

Forage and forage number	Organic matter	Crude protein	Crude fibre	Nitrogen-free extract	Ether extract	Lignin	Calories/gram	
							Dry matter	Organic matter
	%	%	%	%	%	%		
Crested wheatgrass unfertilized – 1	92.7	6.45	33.6	51.2	1.54	8.66	4.47	4.82
Crested wheatgrass top-dressed – 2	94.6	5.30	35.8	51.7	1.66	8.90	4.58	4.84
Intermediate wheatgrass unfertilized – 3	91.2	5.78	35.2	48.6	1.51	8.92	4.37	4.82
Intermediate wheatgrass top-dressed – 4	93.2	6.22	36.1	49.4	1.67	9.38	4.56	4.85
Streambank wheatgrass unfertilized – 5	91.9	7.52	36.3	46.1	2.07	9.18	4.44	4.89
Streambank wheatgrass top-dressed – 6	92.4	8.12	34.9	46.9	2.31	9.15	4.53	4.91
Russian wild ryegrass unfertilized – 7	91.7	8.04	37.1	44.3	1.90	8.73	4.47	4.86
Russian wild ryegrass top-dressed – 8	92.3	9.18	34.6	46.7	2.05	8.92	4.47	4.87
Reed canary grass – 9	90.7	7.92	36.9	44.6	1.39	10.76	4.37	4.85
Tall wheatgrass – 10	91.9	8.58	40.7	41.1	1.86	10.16	4.45	4.83
Alfalfa – 11	90.1	16.84	33.0	38.7	1.69	9.80	4.49	4.94
Mean	90.1	8.16	35.8	46.3	1.79	9.37	4.47	4.86
Se $\bar{x}$	0.67	0.49	1.33	1.17	0.24	0.55	.03	.05
Significant Differences at (P = 0.05)	2>1 and 3 to 11 4>3,9,11 1,6,8>11	11>1 to 10 8>1 to 5 8>1 to 5 6,7,9,10> 1 to 4 5>2 to 4	10>1 to 9 and 11	1,2>5 to 11 3,4>7,10,11 5 to 9>10,11 10>11	6>9	9>1,2,3, 7,8	2>1,3,5 and 7 to 11 4>1,3,5 and 7 to 10 6>5 1,6,7,8,11> 3,9	N.S.

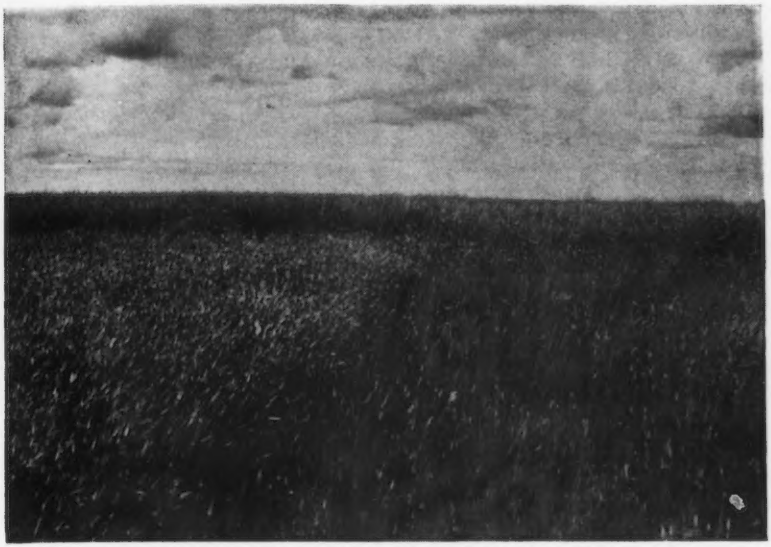

FIGURE 1. Applications of ammonium nitrate fertilizer increased culm formation as indicated by the stand of top-dressed crested wheatgrass (*left*) and unfertilized (*right*).

indicated differences in the nutritive properties of a hay and the refused feed from that hay, a proportional adjustment was made in the percentage content of each chemical fraction for the hay consumed. Statistical analyses of data followed the method outlined by Cochran and Cox (12) for a randomized incomplete-block experiment, while comparisons between means were estimated at the 5 per cent level of significance by the Student-Newman-Keuls multiple range test as discussed by Federer (15).

RESULTS AND DISCUSSION

Chemical Composition of 11 Hays

Proximate principles, lignin, and gross energy determinations suggest four broad groupings for the 11 hays. Crested wheatgrass and intermediate wheatgrass had low crude protein and high nitrogen-free extract contents. Ladak alfalfa was high in crude protein and lignin, but low in nitrogen-free extract and organic matter. A third group, comprised of streambank wheatgrass and Russian wild ryegrass, had crude protein, crude fibre, nitrogen-free extract, and lignin contents approximating the means of the 11 hays. Analyses of reed canary grass and tall wheatgrass indicated near average crude protein, higher than average lignin and crude fibre but fairly low nitrogen-free extract and gross energy contents.

As indicated by data presented in Table 2, crested wheatgrass and intermediate wheatgrass hay from top-dressed fields had higher organic matter contents than the same species from unfertilized fields; this could

TABLE 3.— DIGESTIBILITY OF DRY MATTER, PROXIMATE PRINCIPLES AND LIGNIN OF 11 HAYS

Forage and forage number	Dry matter	Crude protein	Crude fibre	Nitrogen-free extract	Ether extract	Recovery of lignin in feces	Digestible energy	Total digestible nutrients	Starch equivalent
	%	%	%	%	%	%	%	%	%
Crested wheatgrass unfertilized – 1	60.5	50.0	55.2	69.7	45.6	99.6	55.1	59.3	39.0
Crested wheatgrass top-dressed – 2	58.8	43.5	53.0	67.3	37.8	100.5	56.2	57.0	36.0
Intermediate wheatgrass unfertilized – 3	58.3	41.1	57.0	68.1	38.0	96.7	57.9	57.1	36.7
Intermediate wheatgrass top-dressed – 4	58.6	49.4	55.1	66.6	32.9	95.1	56.9	57.9	36.4
Streambank wheatgrass unfertilized – 5	58.9	51.7	61.7	64.1	33.7	102.0	57.0	57.5	35.5
Streambank wheatgrass top-dressed – 6	58.7	55.8	59.4	62.9	36.7	100.4	56.9	57.3	35.4
Russian wild ryegrass unfertilized – 7	61.2	55.9	62.7	66.2	40.9	97.3	59.9	58.9	37.1
Russian wild ryegrass top-dressed – 8	60.4	59.4	58.1	65.6	33.4	100.5	57.2	58.4	37.5
Reed canary grass – 9	56.9	60.3	54.4	63.5	35.3	97.3	55.7	54.7	33.0
Tall wheatgrass – 10	56.2	62.9	56.6	56.4	42.8	95.4	54.4	52.7	29.0
Alfalfa – 11	63.1	76.2	45.5	74.0	36.9	91.2	61.3	57.8	37.8
Mean	59.2	55.1	56.2	65.9	36.7	97.8	57.1	57.1	35.8
Se $\bar{x}$	2.7	3.67	3.95	3.26	11.31	7.76	3.76	2.66	2.74
Significant Differences at (P = 0.05)	N.S.	11>1 to 10 10>1 to 5 6 to 9>2,3	5,6,7>11	11>6,10 1,2,3>10	N.S.	N.S.	N.S.	N.S.	N.S.

be an indication of advanced maturity as Heinrichs and Carson (20) observed increased organic matter content with advanced stages of growth. The percentage content of crude fibre, nitrogen-free extract, ether extract, and lignin were similar for the same grass from both top-dressed and unfertilized stands.

Although the statistical analyses indicated no significant differences in crude protein content between the top-dressed and unfertilized hay of any one species, nevertheless the chemical analyses suggest a higher crude protein content for top-dressed hay of Russian wild ryegrass, streambank wheatgrass, and intermediate wheatgrass. However, crested wheatgrass did not conform to the pattern of the other species, as hay from its unfertilized stand analysed higher for crude protein than hay from its top-dressed field—a suggested explanation for this result is that topdressing increased culm formation; field notes support this observation. (Refer to Figure 1).

Gross energy analyses are reported as Calories per gram in Table 2 for dry matter and organic matter. When calculated on the basis of dry matter, hay from the top-dressed fields had higher gross energy ratings than hay from unfertilized fields; in three of the four species the differences were statistically significant. However, when gross energy coefficients were calculated on the basis of organic matter content of the feeds, the differences were very small and in no case statistically significant; these results indicate that the lower ash content was accountable for the higher gross energy rating of the dry matter of hay from top-dressed fields.

Digestibility Coefficients of 11 Hays

There were few differences in the digestibility coefficients of the 11 hays; all approximate those reported by Schneider (25) for similar feeds.

The digestibility of crude protein follows a definite pattern. Hay having a high percentage of crude protein also rated high for digestible crude protein, but a low percentage digestible crude protein was recorded when the percentage crude protein content of the hay was low. The relationship follows a straight-line regression and according to the following equation:

$$Y = -2.79 + 0.9314\,X.(S\bar{x} = 0.0284).$$

When X = estimated % crude protein in hay ($5.3 \leq X \leq 16.8$)

Y = estimated % digestible crude protein in hay.

Despite higher organic matter, nitrogen-free extract, and ether extract contents of hay from top-dressed fields (Table 3), dry matter digestibility coefficients were lower than those of hays from unfertilized fields. The influence of higher digestibility of the crude fibre and nitrogen-free extracts of unfertilized hay more than compensated for the higher digestibility of crude protein from top-dressed fields. Thus, in three of the four comparisons the hay from unfertilized fields had a slightly higher dry matter digestibility than the same species from the top-dressed fields; the differences were not statistically significant.

Lignin recovery from feces was relatively satisfactory except from Ladak alfalfa. For all the other feeds, whether or not top-dressed, lignin recovery was comparable to that reported for grass hays by Ellis *et al.*

TABLE 4.—AVERAGE DAILY CONSUMPTION AND WEIGHT OF LAMBS

Forage and forage number		Feed (dry matter)	Digestible crude protein	Total digestible nutrients	Digestible energy	Starch equivalent	Average weight of lambs
		grams	grams	grams	Calories	grams	pounds
Crested wheatgrass unfertilized	– 1	700.5	22.4	413.4	1712	272.4	70.3
Crested wheatgrass top-dressed	– 2	674.8	15.7	384.6	1799	244.6	70.8
Intermediate wheatgrass unfertilized	– 3	648.3	15.1	355.4	1610	228.7	68.6
Intermediate wheatgrass top-dressed	– 4	605.4	19.3	346.6	1579	218.0	68.3
Streambank wheatgrass unfertilized	– 5	681.4	26.6	400.7	1771	247.5	69.8
Streambank wheat grass top-dressed	– 6	704.6	32.7	416.7	1864	258.0	70.5
Russian wild ryegrass unfertilized	– 7	820.7	37.2	481.8	2147	305.1	70.6
Russian wild ryegrass top-dressed	– 8	808.0	44.2	482.6	2099	307.5	72.1
Reed canary grass	– 9	482.2	24.4	258.7	1184	156.8	68.0
Tall wheatgrass	– 10	521.4	28.3	262.9	1218	142.6	68.2
Alfalfa	– 11	757.9	100.3	446.4	2091	293.3	67.5
Mean		673.2	33.3	386.4	1734	243.1	69.5
Se $\bar{x}$		70.4	8.96	50.2	232	38.7	3.5
Significant Differences at (P = 0.05)		7,8,11>9,10	11>1 to 10	7,8,11>9,10	7,8,11>9,10	7,8,11>9,10 1>10	N.S.

(14). The poorer recovery of lignin from alfalfa cannot be explained, particularly as Pigden and Stone (24) report little or no change in alfalfa lignin during digestion when estimated by aldehyde yields. However, Adolph *et al.* (1) report lignin recovery from alfalfa feces to be about 95 per cent, a somewhat greater recovery than reported in this paper.

There was a close but by no means exact relationship between total digestible nutrients and digestible energy. Two hays particularly did not conform to the pattern established by the others. Unfertilized crested wheatgrass had one of the lowest ratings for digestible energy and one of the highest when its nutritive value was estimated on the basis of total digestible nutrients, whereas alfalfa had an average rating on the basis of total digestible nutrients but an extremely high rating when estimated by digestible energy contents. Starch equivalent estimations approximate those for total digestible nutrients more closely than for digestible energy values.

Average Daily Intake of Dry Matter, Digestible Crude Protein, Total Digestible Nutrients, Starch Equivalents, and Digestible Energy; and Average Weights of 5 Wethers

The first important point indicated by data presented in Table 4 is the low voluntary consumption of reed canary grass and tall wheatgrass. No matter whether dry matter, total digestible nutrients, starch equivalents, or digestible energy is the measuring unit, the apparent intake of these two feeds is considerably less than for all others, and significantly less than for Russian wild ryegrass and alfalfa. No significant differences of voluntary intake were recorded between hays from top-dressed and unfertilized fields, apparently consumption being determined by species characteristics rather than the fertilizer treatment.

As gains or losses of sheep were small on all feeds, and as there were no significant differences in the average weight of lambs between treatments, the mean intake of nutrients was considered to approximate a satisfactory maintenance ration for 70-pound wethers stalled continuously in digestion cages, at least in so far as the energy producing fractions were concerned. Briggs *et al.* (8) reports a satisfactory feed-lot ration for 70-pound wethers of 260 grams of starch equivalents daily, but when only 185 grams were provided the lambs lost condition and weight. Guilbert and Loosli (17) suggest that a maintenance ration for a 70-pound wether should provide 400 to 500 grams of total digestible nutrients daily. According to these norms the intakes of Russian wild ryegrass, alfalfa, crested wheatgrass, and streambank wheatgrass were sufficient to provide near minimum or more than minimum energy supplying nutrients. Alternately insufficient energy producing nutrients were provided by the voluntary intakes of intermediate wheatgrass, tall wheatgrass, and reed canary grass. The trend in the daily weight pattern of the wethers while on the different feeds (Figure 2) correspond to the above observations.

According to Blaxter and Mitchell (6) a maintenance ration for 70-pound lambs should provide replacement for 13.5 grams of endogenous protein daily, while Guilbert and Loosli (17) suggest that 10 per cent of the total digestible nutrients should consist of digestible crude protein.

TABLE 5.—RELATIVE CONTENT OF TOTAL DIGESTIBLE NUTRIENTS, STARCH EQUIVALENTS, DIGESTIBLE ENERGY AND DIGESTIBLE CRUDE PROTEIN, AS FED IN 11 HAYS, TOGETHER WITH THE RELATIVE INTAKE OF THE SAME STANDARDS

Forage	Relative Concentration of:				Relative Intake of:			
	Total digestible nutrients	Starch equivalents	Digestible energy	Digestible crude protein	Total digestible nutrients	Starch equivalents	Digestible energy	Digestible crude protein
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Crested wheatgrass unfertilized	104	109	95	66	107	112	99	67
Crested wheatgrass top-dressed	100	101	105	48	100	101	106	47
Intermediate wheatgrass unfertilized	100	103	99	49	92	94	95	45
Intermediate wheatgrass top-dressed	101	102	101	65	90	90	90	58
Streambank wheatgrass unfertilized	101	99	100	81	104	102	99	80
Streambank wheatgrass top-dressed	100	99	99	96	108	106	104	98
Russian wild ryegrass unfertilized	103	104	104	94	125	126	127	112
Russian wild ryegrass top-dressed	102	105	100	113	125	126	119	133
Reed canary grass	96	92	96	105	67	65	68	73
Tall wheatgrass	92	81	94	114	68	59	73	85
Alfalfa	101	106	102	269	116	121	120	301
Mean	100	100	100	100	100	100	100	100

Woodman (30) points out that 18 grams, and Smuts and Marais (26) report that 20 grams of digestible crude protein will provide a daily maintenance ration. In view of these norms, and in view of the expectation of nitrogen equilibrium in healthy animals of nearly constant weight (18), it is assumed that minimum or near minimum quantities of digestible crude protein were provided by all feeds.

Swift (27) reports that 1 pound of total digestible nutrients will equal 2018 Calories of digestible energy. Calculations from data reported in Table 4 show that the relationship between the respective means of these feeding standards is 1 pound of total digestible nutrients to 2035 Calories of digestible energy. This is considered to be a close relationship in view of: (a) the few feeds included in the experiments; (b) the slightly higher calorific values of feeds as estimated by a peroxide-bomb type than of those established by an oxygen-bomb type calorimeter (3).

Relative Percentage Contents of Nutrients on Unit and Intake Bases

Calculations presented in Table 5 are relative concentrations of nutrients in the 11 feeds (Columns 1 to 4), and relative concentration of nutrients x voluntary consumption (Columns 5 to 8). All are expressed in reference to a mean equalling 100.

In so far as the relative concentrations of total digestible nutrients, starch equivalent, or digestible energy are concerned, there are small differences between unit weights of the 11 hays (Columns 1 to 3). Strangely enough the classification of the feeding value of these hays would be to place reed canary grass and tall wheatgrass at the bottom of the list when rated on energy supplies (Columns 1 to 3), and near the top of the listing if digestible crude protein is considered the standard of quality (Column 4).

However, when the influences of intake are added to the comparisons based on unit weights, a more definite pattern is evident. Then, no matter which standard is chosen, it is apparent that Russian wild ryegrass hay places first amongst those studied as its intake provided a balance between energy and protein fractions, and more nutrients than required for maintenance. Alfalfa also rates a high position in the comparison. On the other hand, the intake of intermediate wheatgrass, reed canary grass, and tall wheatgrass was insufficient to assure maintenance rations no matter which feeding standard was employed to judge their quality. All of these data indicate that apparent acceptability or palatability is an important factor in evaluating the feeding value of a forage. They support the hypothesis suggested by Crompton (13) ".....the feeding value of a forage depends primarily on the magnitude of its contribution toward the daily energy needs of an animal. Differences in this respect between forages are almost completely a consequence of the relative amounts in which they are consumed".

TRENDS IN LAMB WEIGHTS

Although no significant differences were established in the mean weights of the lambs while on the different feeds (Table 4), there were

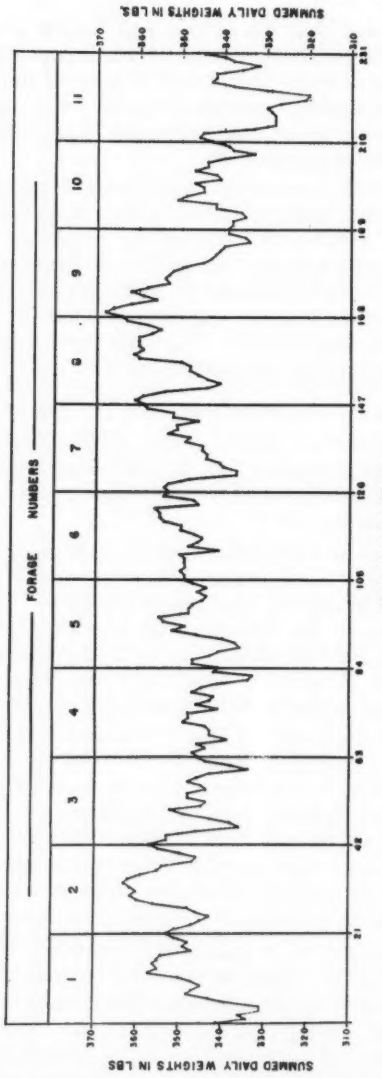

FIGURE 2. Summed daily weight of 5 wether lambs during 11 consecutive 21-day feeding periods. Note the increasing weight trend while on feeds numbered 1, 2, 5, 6, 7 and 8, and the decreasing weights while on feeds numbered 3, 4, 9 and 10. (Forage Numbers as in Table 1).

certain weight trends evident when the daily weights of the wethers were plotted. The sums of the successive daily weights of the 5 lambs while on each feed, irrespective of periods, are plotted in Figure 2.

Notwithstanding the day-to-day variations, the upward or downward weight trends compare favourably to the relative intakes of nutrients (Table 5). The lambs increased their weights when on crested wheatgrass, streambank wheatgrass, and Russian wild ryegrass, and lost weight while being fed intermediate wheatgrass, reed canary grass, and tall wheat-

grass. The weight trend was indefinite during the 21 days while the lambs were fed alfalfa, and it is suggested that the vastly different nutrient composition of alfalfa, compared to that fed previously, necessitated a major shift in the active groups of rumen microflora before balanced metabolic activities could resume (16, 28). These data indicate the possibility that the pattern of daily weights on different feeds could be of importance when assessing nutritive values of feeds. When crested wheatgrass, streambank wheatgrass, and Russian wild ryegrass were fed, well defined cyclic patterns of the summed daily weights were established, but broken and indeterminate patterns characterized the summed daily weights while on the other feeds.

CONCLUSIONS

The several tests applied have demonstrated that certain hays which can be grown in southern Saskatchewan have higher feed value ratings than others. Although some inconsistencies in the results cannot be explained, nevertheless on the basis of chemical analyses, calculated nutritive standards, voluntary consumption, and trends in daily weights, it is apparent that the order of feeding value of the grass hays (when fed alone and irrespective of fertilizer treatment) is as follows: (1) Russian wild ryegrass; (2) crested wheatgrass and streambank wheatgrass (equal); (3) intermediate wheatgrass; (4) tall wheatgrass; and (5) reed canary grass.

Top-dressing with ammonium nitrate fertilizer increased percentage crude protein and apparent digestibility of the crude protein slightly, but did not increase the digestibility of the dry matter.

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SYMPTOMS OF SOUR CHERRY YELLOWS AND NECROTIC RING SPOT IN RELATION TO TIME OF INOCULATION[1]

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ABSTRACT

Each month throughout the growing seasons of 1954 and 1955 two pairs of virus-free Montmorency sour cherry trees were inoculated by budding or patch grafting, one pair with cherry yellows and the other with necrotic ring spot virus. Shock symptoms induced by the two viruses were indistinguishable except that growth was retarded more severely and longer with yellows than with ring spot. However, the type and distribution of initial symptoms varied with the time of inoculation. Four symptom patterns were distinguished, and each was associated with an inoculation period. Seasonal symptom variations also revealed that the rate of movement of the yellows virus differed from that of the ring spot virus.

INTRODUCTION

Sour cherry yellows (3) and necrotic ring spot (1), the two principal virus diseases of sour cherry in Ontario, are widespread within most bearing orchards. Since 1950 the use of virus-free bud wood has greatly improved the quality of sour cherry nursery stock in New York State (2). Data obtained by the authors in 1957[3] indicated that this method has given similar results in Ontario. Hence many orchards now being planted contain a high percentage of virus-free trees. The need for the development of methods to control the spread of virus in these new orchards is obvious. The rate and pattern of spread as reported by Willison, Berkeley and Chamberlain (5), suggest an arthropod vector. However, neither the season of greatest natural spread nor the agent responsible for it is known. Accordingly, experiments were conducted to determine if the time of inoculation had any effect upon the subsequent symptom expression. If a relationship between these factors were established it should be possible to determine the time of year during which natural spread occurred. This information would be helpful in the search for the vector(s) and in devising control measures.

MATERIAL AND METHODS

Virulent strains of the cherry yellows and necrotic ring spot viruses, designated Y. 2 and N. 5 respectively, were used throughout these experiments. These strains induce strong, recurrent symptoms. In 1956, Willison, Weintraub and Ferguson (6) showed by electron microscopy that Y. 2 and N. 5 were pure strains of the cherry yellows and the necrotic ring spot viruses respectively. These viruses, each from a single source, were transferred by bud or patch graft to healthy 3-year-old Montmorency sour cherry trees on or about the 15th day of each month, in August in 1953, from April to October in 1954, and from April to September in 1955.

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Price (4) found that the site of inoculation influenced the severity of curly top in tobacco. It seemed probable that the site of inoculation with cherry yellows and necrotic ring spot viruses might also affect symptom expression and distribution in young trees. Hence, three inoculation procedures were followed: (a) two buds were placed close together on the main trunk below the point of branching; (b) four or five buds were placed on as many secondary branches at points 1 or 2 feet from the terminals; and (c) in the 1953–54 series only two buds were placed on single secondary branches. In all cases inoculations were made to pairs of trees and each series included uninoculated controls.

RESULTS AND DISCUSSION

Shock and Recurrent Symptoms

The cherry yellows and necrotic ring spot viruses are both capable of inducing an initial severe symptom phase, known as "shock", which appears to be the reaction of previously healthy parts of a tree to invasion by a virus during a time of rapid tree growth. Shock, if it develops, occurs at the first bud-break following infection. It is characterized by a delayed foliation that may be either general or confined to only a portion of a given tree. In the delayed sector of the tree the first leaves are smaller than normal and many develop brown necrotic spots or rings of varying intensities. The necrotic areas frequently drop out so that the leaves are ragged. This condition is apparent only at the beginning of the growing season as subsequent leaves develop normally and obscure the initial symptoms.

The only difference observed between shock caused by Y. 2 and that by N. 5 was in the degree of growth retardation. Shock always delayed foliation but trees in shock with N. 5 soon recovered and by the end of the season terminal elongation of branches and development of lateral shoots were nearly equal to those of the uninoculated controls. On the other hand, trees infected with Y. 2 grew very little in the year of shock; terminal growth was only 25 to 50 per cent of that in the controls and the development of laterals was far below normal.

Recurrent secondary symptoms appeared in subsequent years on trees inoculated with Y. 2 and N. 5 as shown in Figure 1. Y. 2 caused the typical yellow-leaf symptom during late June followed by the casting of affected leaves. N. 5 caused a relatively inconspicuous etching on the first leaves that developed each spring. This symptom consisted of minute rings and partial rings that had a water-soaked appearance. The surfaces of affected leaves were roughened but no necrosis occurred. Certain mild strains of the ring spot virus do not induce recurrent etch symptoms.

Inoculation Time—Symptom Relationship

Each of the four inoculation periods, namely, April, May to July, August, and September and October, was associated with a distinctive combination of symptom distribution and type. Shock symptom distribution patterns characteristic of these periods are shown in Figure 2.

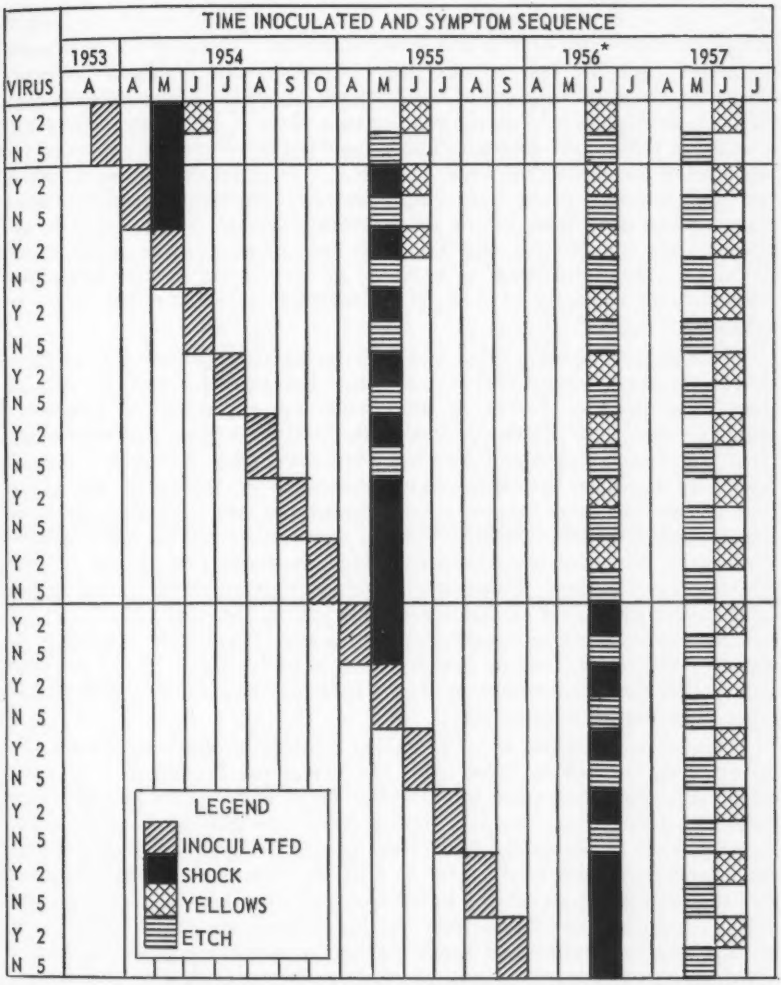

FIGURE 1. Types and succession of virus symptoms. *Because the 1956 spring was very late, shock and etch symptoms, which usually develop in May, did not appear until early June.

Trees that were inoculated in April developed shock symptoms at bud-break a few weeks later (Figure 1). The time of appearance and progress of symptoms were identical for Y. 2 and N. 5. Trees inoculated on the branches developed symptoms first on those parts above the point of budding, then on parts below this point along a direct path to the base of the tree without affecting side branches. Leaf buds that were just opening at the time of the downward movement of the virus reacted with shock and thus indicated the progress of infection. Later upward movement

took place after the first leaves had fully opened and hence no further symptoms developed during that growing season. Initial shock symptoms suggested that the two viruses had followed similar invasion patterns. However, the outer portions of the branches of the Y. 2-infected trees that did not develop shock in the first season showed slight to mild shock symptoms the second season. This secondary shock can be explained on the basis of incomplete movement of the Y. 2 virus into new growth during the first season. Areas that had previously reacted with shock were symptomless until later in the season, when typical yellowed leaves appeared. On the other hand, there was no indication of a second shock phase on trees inoculated with N. 5; all developed typical secondary symptoms in the form of a slight to moderate etching of the leaves in subsequent years (Figure 1).

Inoculation of cherry trees with Y. 2 during May, June or July resulted in general shock symptoms at bud-break the following spring. Site of inoculation had no observable effect upon the expression as symptoms appeared uniformly above and below inoculation points. Apparently the virus was unable to enter buds that were developing during this period. Also it appears that the virus was not present in the top of the tree at the time of bud maturity because no symptomless areas such as occurred in the August inoculants described below were obtained in these groups. Presumably the virus moved downwards to the roots, and did not become systemic until spring. However, trees inoculated with N. 5 during the same period developed secondary etch symptoms the next season without any observable shock or retardation of growth. Presumably this virus, in contrast with Y. 2, became systemic before bud break. Thus, it would appear that the shock symptom of ring spot may not occur when infection takes place during the summer.

August inoculations with Y. 2 also resulted in shock symptoms the following spring, but in these trees the inoculated branch and, in some cases, adjacent parts were symptomless (Figure 2). This suggests that the virus moved from the inoculation point into the surrounding tissue before growth ceased in the fall. Results with N. 5 were variable in that they were comparable to those for Y. 2 in two series, but in the 1954 series only etch symptoms developed in the spring (Figure 1); the latter indicates that if conditions are favourable the N. 5 virus can move rapidly and become systemic before bud break so that no shock symptoms appear.

Late fall inoculations were identical for both viruses in that severe shock symptoms developed throughout the trees the following spring.

Trees inoculated on the trunk below the point of branching reacted like those inoculated on the branches as far as type of symptom was concerned. However, the distribution of these symptoms varied as much between trees within treatments as between treatments. Thus, trunk-inoculated trees did not fit into the above scheme.

The value of the results reported here will ultimately depend upon how well the symptom patterns of naturally infected trees fit those obtained in these tests. Although it seems safe to assume that natural infection is usually the result of branch or leaf inoculation as opposed to trunk or

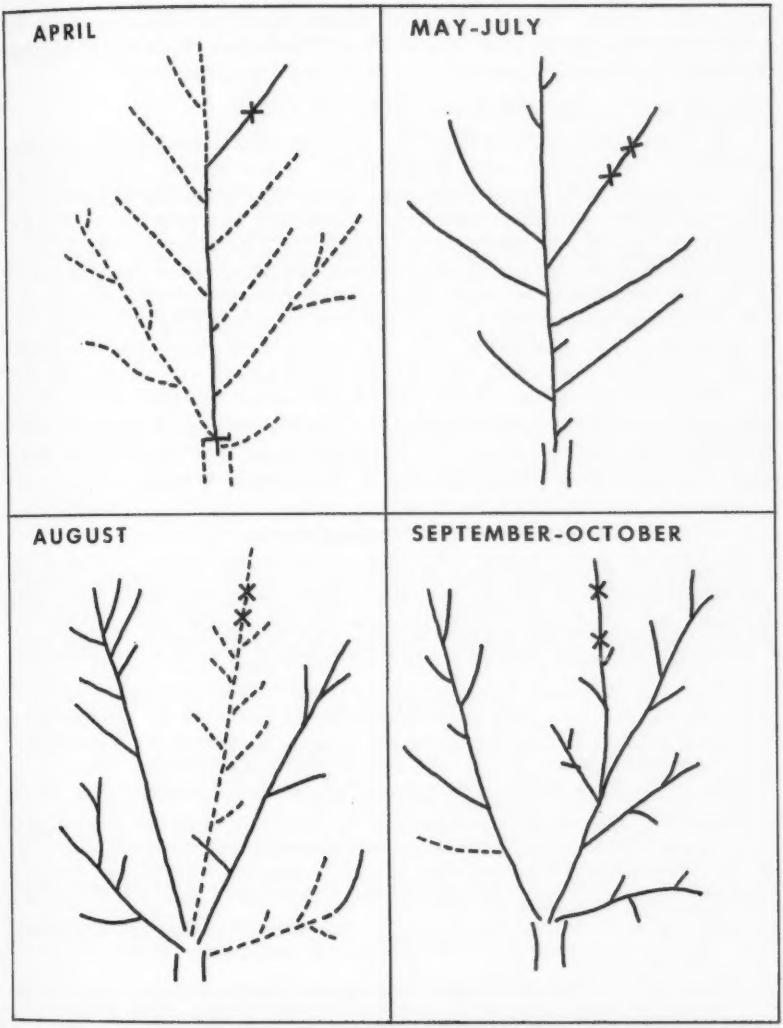

FIGURE 2. Diagrammatic representation of the effects of time of inoculation with cherry yellows or necrotic ring spot viruses on shock symptom patterns induced in Montmorency sour cherry trees.
x, point of inoculation; —, symptom-bearing branch; ---, symptomless branch.

root inoculation, this is not known for certain. Nor is it known whether vector-inoculated trees react in the same way as graft-inoculated ones. Studies are being made of the initial symptom patterns of naturally infected trees. If it is found that these can be fitted into the above groupings then it should be possible to define the time of year when natural spread most

frequently occurs. This information would be of considerable value in organizing tests for vectors and in the development of chemical control programs.

Differential Movement of Y. 2 and N. 5

The above observations show that there is a distinct difference between the movements of the Y. 2 and N. 5 viruses. The reason for this difference was not determined in these tests. However the difference presents further evidence that cherry yellows and necrotic ring spot are caused by related but distinct viruses. It is obvious from the shock patterns that in the spring, except for a very brief period during bud break, the movement of the Y. 2 virus into new growth is retarded or prevented. But as the new wood matures in the fall the plant loses this ability to inhibit the Y. 2 virus. The possibility that temperature or some other climatic factor might be responsible for the results obtained was considered but no correlation could be found. Thus the nature of this inhibition is unknown but its existence explains the distribution of shock symptoms obtained with the Y. 2 virus. In contrast the N. 5 virus seems to move freely through cherry trees during the entire growing season.

ACKNOWLEDGEMENTS

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VARIETAL AND ENVIRONMENTAL EFFECTS ON RAPESEED

II. FATTY ACID COMPOSITION OF THE OIL[1]

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ABSTRACT

The content of C_{16}, C_{18}, C_{20}, C_{22} fatty acids were measured by gas liquid phase chromatography and linoleic and linolenic acids by spectral analyses on the oil from seven varieties of rapeseed grown at seven stations in Western Canada. Significant differences were found between varieties for all oil properties except the content of C_{16} acids. The major variation occurred in C_{18}, C_{22} and linoleic acids with lesser amounts in the C_{20} and linolenic acids. The varieties Golden, Argentine, Regina II and Swedish are classed as high, Gute and Arlo as intermediate, and Polish as low erucic acid oils.

INTRODUCTION

The fatty acid composition of a limited number of oils from rapeseed and mustard seed has been investigated in this laboratory by fractional distillation of 300 to 500 grams of the methyl esters of the fatty acids derived from the oil (1, 5). Each fraction was analysed for the constituent fatty acids by standard procedures. This work indicated that the C_{20} and C_{22} acid fractions were mainly eicosenoic and erucic acids with minor amounts of the saturated and polyethenoid acids of the same chain lengths. The major variation in the fatty acid composition occurred with oleic (C_{18}), linoleic (C_{18}) and erucic (C_{22}) acids whereas the amounts of palmitic (C_{16}), eicosenoic (C_{20}), stearic and linolenic (C_{18}) were relatively constant.

The procedure used in these studies required too much time and too large samples to be used for a varietal and environmental study. An investigation of gas liquid phase chromatography (2) indicated that this technique could be successfully applied to this problem. The data are similar to the results of fractional distillation and coupled with the determination of iodine values and spectral analyses for linoleic and linolenic acids should yield valuable information on the effect of variety and environment on the fatty acid composition of rapeseed oils. The present study was undertaken on the oils from seven varieties of rapeseed grown at seven locations in Western Canada.

MATERIALS AND METHODS

The oils were obtained from seven varieties grown at each of seven stations as described in Part I of this series (4). The oil was saponified, acidified and the free fatty acids were extracted with ethyl ether. Ether was removed by evaporation on a laboratory rotary evaporator under reduced pressure and an aliquot of the acids (100 mgm.) was converted

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TABLE 1.—ANALYSIS OF RAPESEED OIL METHYL ESTERS BY GAS LIQUID CHROMATOGRAPHY*

	Argentine	Golden	Regina II	Swedish	Gute	Arlo	Polish
	Content of C_{22} Acids %						
Mileta	40.1	46.8	43.2	44.2	34.5	30.1	23.5
Morden	43.2	49.2	45.7	47.0	37.2	31.7	24.8
Brandon	40.8	45.7	46.6	43.8	37.0	32.8	27.5
Winnipeg	45.3	47.6	43.8	43.4	31.2	32.9	25.2
Swift Current	40.5	47.3	44.2	46.5	31.9	31.0	23.1
Lethbridge	40.0	45.5	46.4	43.7	37.0	31.1	22.4
Agassiz	40.4	49.0	44.1	47.1	33.7	29.8	22.8
	Content of C_{20} Acids %						
Mileta	13.6	14.2	14.1	13.3	14.4	12.6	13.0
Morden	13.7	13.7	13.3	12.4	14.9	11.8	13.2
Brandon	13.8	15.1	14.7	12.4	11.9	12.2	12.2
Winnipeg	14.3	13.5	13.1	13.0	12.3	12.5	12.8
Swift Current	13.6	14.8	12.1	12.9	15.4	13.5	13.3
Lethbridge	14.4	15.1	15.1	13.9	15.7	13.0	13.5
Agassiz	13.4	13.3	13.1	13.0	15.8	13.2	13.7
	Content of C_{18} Acids %						
Mileta	42.7	35.5	38.7	38.3	48.3	53.5	60.1
Morden	39.4	34.0	37.3	36.8	45.3	53.7	58.3
Brandon	41.6	35.3	34.9	39.9	47.4	52.0	57.3
Winnipeg	37.0	35.7	39.9	39.7	53.4	52.0	58.7
Swift Current	42.1	34.2	40.5	37.0	48.3	51.9	60.0
Lethbridge	42.2	36.0	34.9	38.7	44.6	52.9	61.2
Agassiz	42.4	34.8	39.3	36.0	46.7	53.7	60.4
	Content of C_{16} Acids %						
Mileta	3.5	3.6	4.0	4.3	2.8	3.8	3.4
Morden	3.6	3.0	3.7	3.8	2.5	2.8	3.7
Brandon	3.8	3.9	3.8	3.9	3.7	3.0	3.0
Winnipeg	3.4	3.2	3.6	3.9	3.0	2.6	3.2
Swift Current	3.8	3.7	3.1	3.6	4.5	3.5	3.6
Lethbridge	3.4	3.3	3.6	3.7	2.7	3.0	2.9
Agassiz	3.8	2.9	3.4	3.8	3.8	3.2	3.1

* Column 3 feet × ¼ inch with 20–40 mesh C_{22} firebrick with washed silicone grease 4:1 (w/w). Column temperature 240°C., injector temperature 265°C., bridge current 150 m.a., carrier gas—helium at 70 ml. per min.

to methyl esters by addition of an ethereal solution of diazomethane. The solvent was removed from the esters by evaporation under reduced pressure.

Iodine values were measured on the oils by the Wijs method with 1-hour reaction time. The analyses of linoleic and linolenic acids were made according to the standard A.O.C.S. spectral procedure.

The gas liquid chromatographic unit of conventional design was constructed in the laboratory. The silicone grease for the liquid phase was prepared according to the method outlined by Cropper and Heywood (3) and the time required for emergence of the C_{22} methyl esters was 25 minutes.

The area of the peak representing each fraction was calculated by using the height and base of the triangle which was formed by drawing tangents to the curves on the recorder chart. The accuracy of the method was checked by using known mixtures of purified fatty acid esters and agreement within 1 per cent with the calculated compositions was found. The composition as determined by gas liquid phase chromatography (G.L.P.C.) was found to compare favourably with the results of fractional distillation which has been used in other studies*.

The amounts of C_{24} acids revealed in the results were less than 1 per cent and were not included in the present data.

RESULTS

The data from the gas liquid chromatographic analyses (Table 1) are similar to those obtained by fractional distillation in the previous publications (1, 5). This study confirms the earlier observation that the major differences between samples of rapeseed oil is in the C_{18} and C_{22} fatty acid contents, with C_{16} and C_{20} relatively constant. The previous

* *Unpublished data* from this Laboratory.

TABLE 2.—IODINE VALUES AND SPECTRAL ANALYSES FOR LINOLEIC AND LINOLENIC ACIDS IN RAPESEED OILS

	Argentine	Golden	Regina II	Swedish	Gute	Arlo	Polish
	Iodine Values						
Mileta	101.5	103.1	100.9	101.9	105.7	106.5	107.4
Morden	102.0	102.0	97.6	100.7	107.0	107.9	107.7
Brandon	103.2	105.9	99.6	103.9	102.4	108.2	107.1
Winnipeg	102.9	102.8	100.9	100.8	107.7	107.7	108.9
Swift Current	102.5	101.8	102.7	100.7	104.9	104.7	105.3
Lethbridge	101.7	101.8	99.2	99.4	103.4	103.8	104.1
Agassiz	104.6	103.8	102.1	102.5	100.2	105.8	107.3
	Linoleic Acid %						
Mileta	10.6	17.2	14.9	15.0	18.0	19.8	18.9
Morden	11.0	15.6	16.2	17.2	15.1	18.1	18.1
Brandon	15.8	13.8	12.4	13.5	15.0	16.8	18.1
Winnipeg	15.1	13.6	10.4	12.3	17.5	19.2	19.0
Swift Current	16.0	10.6	14.4	11.0	16.7	18.7	18.7
Lethbridge	12.5	16.4	12.1	12.2	16.4	17.2	17.7
Agassiz	12.5	15.5	16.8	13.1	19.7	17.9	18.3
	Linolenic Acid %						
Mileta	9.0	8.7	7.8	7.7	7.7	7.2	7.2
Morden	7.0	8.2	7.2	7.4	6.2	7.9	8.9
Brandon	8.6	5.7	6.9	8.2	6.6	7.5	8.7
Winnipeg	7.5	6.2	5.4	6.5	8.3	7.5	8.4
Swift Current	7.6	7.4	7.8	7.3	7.2	6.2	7.6
Lethbridge	7.4	8.6	5.6	7.3	6.9	6.0	7.6
Agassiz	9.3	9.1	6.9	9.4	6.4	7.9	9.1

TABLE 3.—STATION AND VARIETAL MEANS OF RAPESEED OIL PROPERTIES

	Gas liquid chromatographic analyses				Iodine value	Spectral analyses	
	C_{22}	C_{20}	C_{18}	C_{16}		Linoleic	Linolenic
	Station Means						
Mileta	37.5	13.6	45.3	3.6	103.9	16.3	7.9
Morden	39.8	13.3	43.5	3.3	103.6	15.9	7.5
Brandon	39.2	13.2	44.1	3.6	104.3	15.0	7.4
Winnipeg	38.5	13.1	45.2	3.3	104.5	15.3	7.1
Swift Current	37.8	13.7	44.9	3.7	103.2	15.2	7.3
Lethbridge	38.0	14.4	44.4	3.2	101.9	14.9	7.1
Agassiz	38.1	13.6	44.8	3.4	103.7	16.3	8.3
95% Fiducial limits	± 1.3	± 0.6	± 1.4	±0.3	±1.2	± 1.4	±0.7
	Varietal Means						
Argentine	41.5	13.8	41.1	3.6	102.6	13.4	8.0
Golden	47.3	14.2	35.1	3.4	103.0	14.7	7.7
Regina II	44.9	13.6	37.9	3.6	100.4	13.8	6.8
Swedish	45.1	13.0	38.1	3.9	101.4	13.5	7.7
Gute	34.6	14.3	47.7	3.3	104.5	16.9	7.0
Arlo	31.6	12.7	52.8	3.1	106.4	18.2	7.2
Polish	24.2	13.1	59.4	3.3	106.8	18.4	8.2
95% Fiducial limits	± 1.3	± 0.6	± 1.4	±0.3	± 1.2	±1.4	±0.7

work (1, 5) indicated that the C_{16}, C_{20} and C_{22} fractions were mainly palmitic, eicosenoic and erucic acids respectively. The C_{18} fraction is composed of stearic, oleic, linoleic and linolenic acids and the stearic acid content was shown to be relatively constant for the varieties studied earlier (1, 5). The spectral analysis (Table 2) gives the values for linoleic and linolenic acids and the value for oleic and stearic combined may be obtained by difference from the total C_{18} acids (Table 1).

Examination of the data in Table 2 shows the over-all variation for linoleic acid is from 10.4 to 19.8 per cent or 9.4 units per cent; for linolenic acid the values range from 5.4 to 9.4 or 4.0 units per cent. Taking the high and low values of C_{18} content from Table 1 as 61.2 per cent and 34.0 per cent, and determining the (stearic + oleic) content by difference using the data in Table 2, the variation in (stearic + oleic) is from 35.9 to 10.2 or 25.7 units per cent. Since the content of stearic acid is relatively constant this figure represents the total variation in oleic acid. Similarly the C_{22} content (erucic acid) has a range from 49.2 to 22.4 per cent or 25.2 units per cent. The major variants in fatty acid composition of rapeseed oils are in the amounts of oleic, linoleic and erucic acids.

The station means (Table 3) show little variation whereas there are marked differences in varietal means. This is substantiated in Table 4 where significant differences were found for varieties but not for stations. The varieties in this study can be grouped into classes according to C_{22} (erucic) acid content from the data in Table 3 as follows: *high*—Golden,

TABLE 4.—ANALYSIS OF VARIANCE FOR OIL PROPERTIES MEAN SQUARES

Oil property	Variance due to		
	Variety	Station	Interaction
C_{22}	514.7**	4.80	2.80
C_{20}	2.87**	1.34	0.65
C_{18}	569.0**	2.81	3.42
C_{16}	.243*	.446	.142
Iodine value	40.80**	5.14	2.65
Linoleic	34.89**	2.44	3.56
Linolenic	1.98*	1.38	0.77
Degrees of freedom	6	6	36

* Significant to 5% level
** Significant to 1% level

Swedish, Regina II and Argentine; *intermediate*—Arlo and Gute; and *low*—Polish. The mean for Argentine is significantly lower than for the other three varieties in the same class but is markedly higher than for the intermediate group. It is doubtful if the difference between the Arlo and Gute is significant. However, in a similar classification based on C_{18} content there is a significant difference between these two varieties. The content of linoleic acid can be used in a similar manner but the division would be only into two groups, Argentine and Polish "types", with the latter having the higher value. The difference in iodine values between these two general types of rapeseed of around 4-5 units can be accounted for by the average difference in content of linoleic acid of around 3-5 per cent.

DISCUSSION

The variation in C_{22} (erucic) acid content is the most interesting section of this investigation since this acid has been the subject of a number of nutritional studies. It is obvious that there is a wide range of C_{22} (erucic) contents in the present varieties of rapeseed and it should be possible by a breeding and selection program to produce varieties with a desired level for either edible or inedible uses.

The use of gas liquid chromatography provides a rapid method of determining erucic acid content using a small amount of oil. This property is the most reliable in assessing the type of oil and is the main concern in the industrial usage of the oil. It is evident that a measure of iodine value is not a reliable index of fatty acid composition since the interpretation of this value is complicated by the presence of monoethenoid acids of varying chain length. A comparison of the oil from Argentine rapeseed at Agassiz (I.V. 104.6) and Polish from Lethbridge (I.V. 104.1) shows erucic acid contents of 40.4 and 22.4 per cent respectively. Similarly Golden from Brandon (I.V. 105.9) and Arlo from Agassiz (I.V. 105.8) have C_{22} contents of 45.7 and 29.8 per cent respectively.

The lack of significant variation between stations may be due to the small number of stations and the location of these stations in the southern areas of the provinces. Further investigations are required

which would include stations in the Park Belt and Grey Soil zones to complete the effect of environment on fatty acid composition. The present data suggest that varietal effects are greater than environmental and that oil with a preferred fatty acid composition could be obtained by selection of a suitable variety.

ACKNOWLEDGEMENT

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THE PRECISION OF FIELD EXPERIMENTS WITH VEGETABLE CROPS AS INFLUENCED BY PLOT AND BLOCK SIZE AND SHAPE

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ABSTRACT

In 1956 weights of marketable ears of hybrid sweet corn from three irrigated uniformity trials were recorded to determine the effect of varying plot and block sizes and shapes on yield variability. The coefficients of variation decreased with increased plot size. For block shapes of 1 x 6 plots (1 plot long and 6 plots wide) and block shapes of 3 x 2 plots (3 plots long and 2 plots wide) widening the plots was more effective in reducing variation than lengthening them. However, for block shapes of 6 x 1 plots and block shapes of 2 x 3 plots lengthening the plots was most effective. Estimates of cost were obtained showing the percentage of the total cost that was proportional to the number of plots used and the percentage that was proportional to the total area used per treatment. Optimum plot size for yield and quality studies was found to be one of two basic units (10 ft. by 6 ft., or 20 ft. by 3 ft.) depending upon cost assumed and the value of the regression coefficient. Smith's method for determining optimum size of plot agreed with the coefficients of variation method as to optimum size of plot.

INTRODUCTION

In the realm of field research with vegetable crops it is difficult to find adequate information regarding optimum plot and block sizes and shapes. This means that wide ranges of plot and block sizes and shapes have been chosen arbitrarily and, in many instances, without adequate information. Such important factors as soil heterogeneity and cost have not always been considered seriously when vegetable crops experiments have been designed. Thus, reduced plot efficiency has accompanied many experiments in this field of research.

The uniformity trial has been most useful in determining yield variability as affected by plot and block size and shape. The reason for this, as given by Cochran (4), is that neighbouring units can be amalgamated to form large plots of various sizes and shapes. The variation in yield over the field due to soil heterogeneity and other factors can be calculated for any size and shape of plot formed. Once the optimum size and shape of plot has been determined, the standard deviation of each plot and number of replications required to reach a given degree of accuracy in the comparison of the mean treatment yields can be calculated.

This study was primarily concerned with the utilization of data from uniformity trials with vegetable crops for the estimation of optimum plot size and shape. This paper deals with data from sweet corn uniformity experiments at three locations in southern Alberta.

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LITERATURE REVIEW

A study of the literature shows a limited amount of information regarding the yield variability of vegetable crops as affected by plot size and shape. Only one paper (9) on this subject includes research on sweet corn. It was, therefore, desirable to review the methods and results of research with other crops.

The early workers (circa 1931) were primarily concerned with finding the most efficient plot size without considering cost. The coefficient of variation was the statistic used to show that efficiency generally decreased as plot size increased.

Bryan (1), working with field corn, and Loesell (8), working with white pea beans, found that, although small plots resulted in greater precision, there is a practical minimum below which handling of the plots becomes too difficult. Christidis (3), was the first to emphasize the importance of plot shape as well as size. He theorized that long plots constitute the only means of reducing the effect of soil heterogeneity. By decreasing the width of plots and correspondingly increasing their length the productivity of the individual plots becomes more uniform. These findings were substantiated by the work of Justesen (6) and Kalamkar (7) working with potatoes. Justesen (6) pointed out that the reason for the increased efficiency of the longer rows is due to the partitioning of local differences so that they become distributed over different plots.

Immer (5) states that optimum size and shape of plot for determining yield in crops is not necessarily the most efficient for quality determination or vice versa. Such factors as relative cost of planting, cultivating, and harvesting also should be considered when determining the optimum size and shape of plot. However, he did not attempt to estimate the costs involved.

Smith (11) pointed out that data from uniformity trials up to that time had not provided any reliable method for determining the best size of plot for any particular purpose. He proposed that the regression of variability on plot size becomes linear when plotted on logarithmic paper and could be more easily interpreted. This means that the relative reduction in variability for a relative increase of plot size is similar throughout the observed range, i.e., doubling plot size always results on the average in the proportional reduction in variability. The relationship was described by the following formula: $Vx = V_1/n^b$ where Vx is the variance of yield per unit area of plot composed of n units of area, V_1 is the variance of yield of the basic plot size, and b is the exponent which measures soil heterogeneity and correlation between adjacent plots. Smith (11) also presented a formula for estimating the minimum cost per plot and thus related cost to size in finding the optimum plot size.

Robinson, Rigney and Harvey (10), working with peanuts, estimated costs by indicating the percentage of the cost that was proportional to the number of plots used and the percentage that was proportional to the total area used per treatment. Assuming a desired degree of accuracy ($S\bar{x} = 3.5$ per cent) the relative optimum cost for various plot dimensions could then be calculated and optimum plot size indicated. They showed

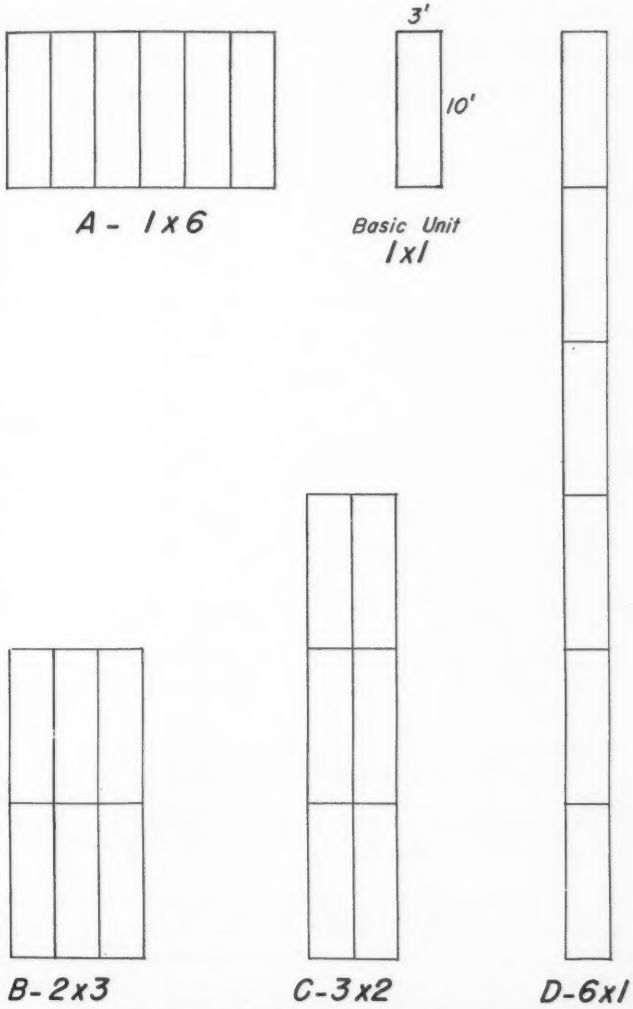

FIGURE 1. Four block shapes were used as follows: *A*—1 plot long and 6 plots wide; *B*—2 plots long and 3 plots wide; *C*—3 plots long and 2 plots wide; *D*—6 plots long and 1 plot wide.

the most efficient plot size when different percentages of the total cost of a treatment are proportional to the total area used. When this cost was relatively low (20 per cent) a range of from 2 to 10 units could be used without deviating appreciably from the optimum. As this cost increased the small plots became the more efficient and the range narrowed. Their cost function, minimized to give the optimum plot size for any degree of soil heterogeneity and any assumption of the distribution of the cost,

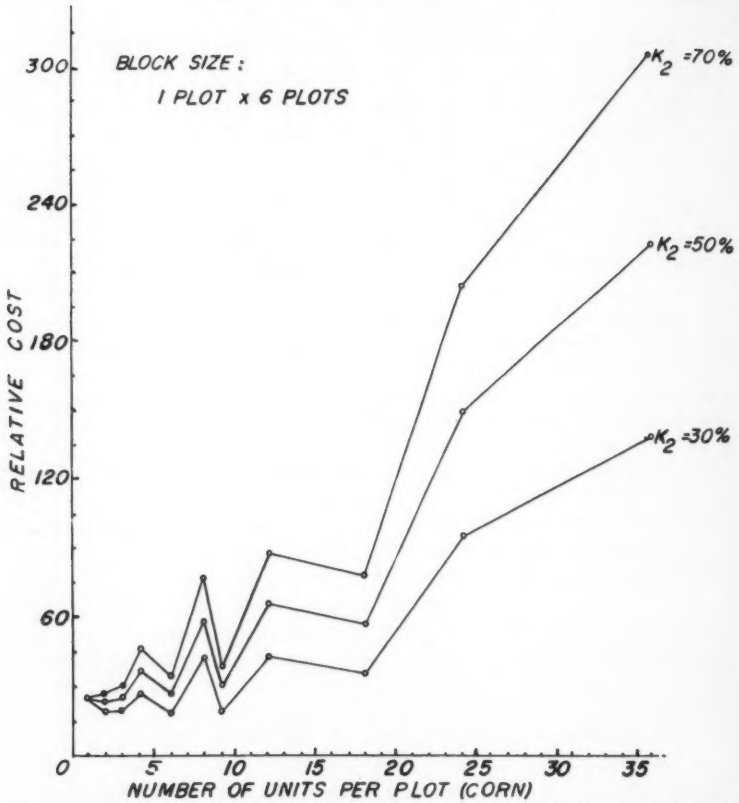

FIGURE 2. The relative cost of obtaining a desired degree of accuracy ($S\bar{x}$ = 3.5 per cent) using different plot sizes and blocks of 1 x 6 plots with sweet corn, assuming various percentages of the total cost to be proportional to the total area used per treatment (K_2).

showed that, when the proportion of cost that is related to plot size is low, small plots are most efficient regardless of soil uniformity. As this cost increases, large plots become the more economical especially on less uniform soil.

Recently Moore and Darroch (9) reported only 10 publications dealing with uniformity trial data on six vegetable crops. They reported that, for sweet corn trials, to obtain significance at the 3.5 per cent level, six replications of 40-foot plots were adequate. Their data, as in previous papers, showed the reliability of the long narrow plot. It was noted by them that block shape effect was not constant for all the crops they tested. Their assumption was that this is more closely related to direction of soil fertility variation than to crops. They concluded that plot and block arrangement with regard to soil fertility variability are most important and failure to compensate for them in planning may result in substantial precision losses.

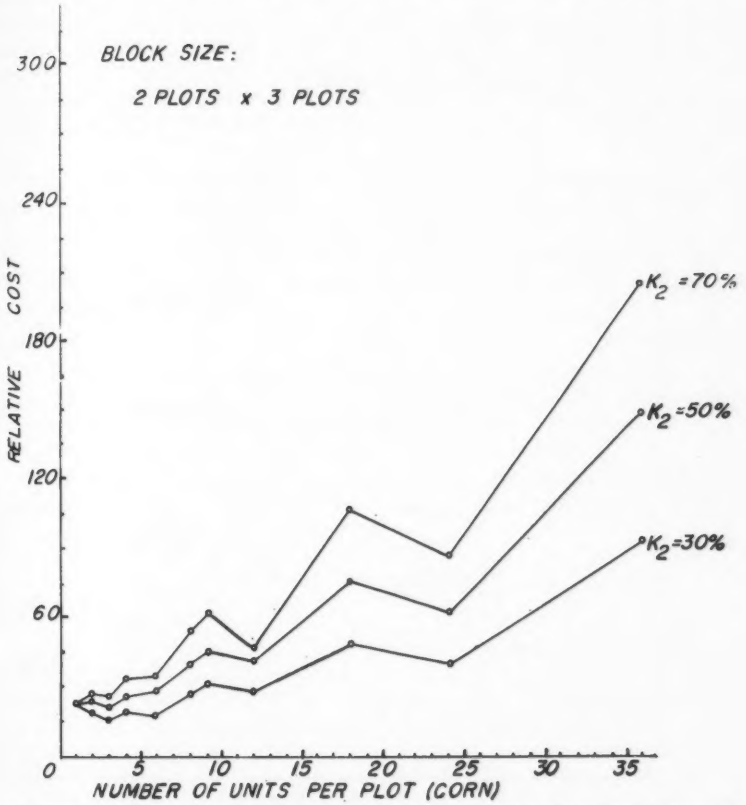

FIGURE 3. The relative cost of obtaining a desired degree of accuracy ($S\bar{x}$ = 3.5 per cent) using different plot sizes and blocks of 2 x 3 plots with sweet corn, assuming various percentages of the total cost to be proportional to the total area used per treatment (K_2).

MATERIALS AND METHODS

Uniformity trials with hybrid sweet corn (var. Seneca Golden) were conducted in 1956 at three locations. Lethbridge, Vauxhall and Cranford were chosen to represent distinctly different soil and climatic conditions. Lethbridge has the coolest climate and the heaviest soil, a silty clay loam; Vauxhall is warmer and the driest location with a sandy loam soil; and Cranford is the warmest with a loam soil. These different locations made it possible to conduct three uniformity trials in one season.

The experimental area at each location consisted of 32 rows, each 180 feet long. Each of the 32 rows was divided into 18 units of 10 feet each, thus giving a total of 576 units. The rows were 3 feet apart and in each row the plants were thinned to 1 foot apart. Two guard rows surrounded the entire experimental area.

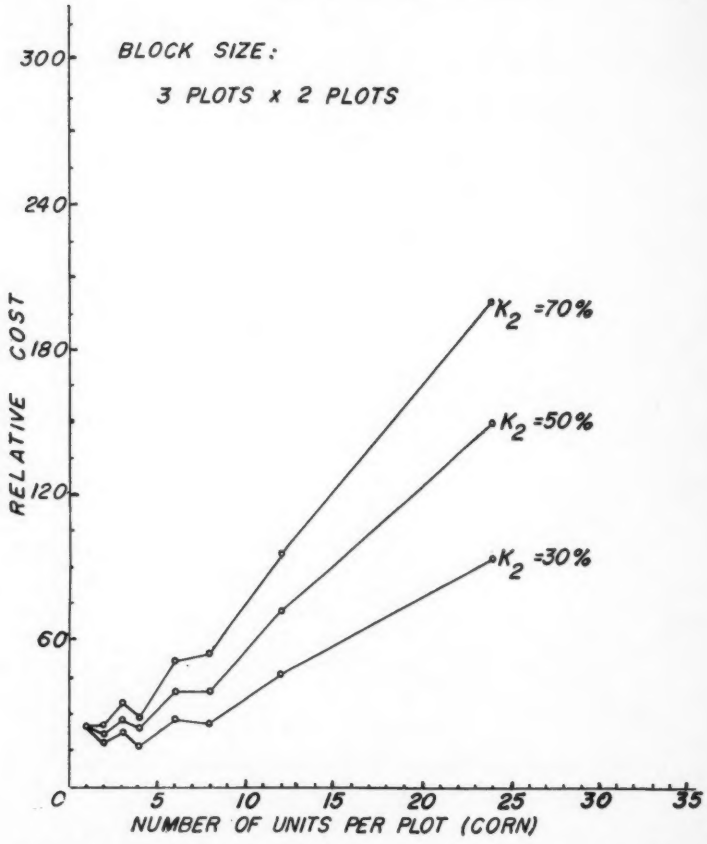

FIGURE 4. The relative cost of obtaining a desired degree of accuracy ($S\bar{x}$ = 3.5 per cent) using different plot sizes and blocks of 3 x 2 plots with sweet corn, assuming various percentages of the total cost to be proportional to the total area used per treatment (K_2).

The 10-foot basic unit was used since there was no reason for reducing the length further; yet it was satisfactory for combining into large sized plots. Furthermore, the weight of kernels from the ears of each 10-foot unit was found, on the average, to be adequate for laboratory, quality, and taste panel tests.

Seeding and Care of Experimental Areas

Relatively uniform, fertile land was selected at each location. At Lethbridge, the experimental area was seeded on land that in the previous year received 70 pounds ammonium-phosphate fertilizer per acre and produced a green manure crop. In the year before, the land produced grain. At Vauxhall, the cropping history was the same as at Lethbridge for the previous 2 years. Previously the land had produced crested

wheatgrass for several years. At Cranford, the experimental area produced wheat the previous year, and tomatoes the year before that. For tomato production the land was fall ploughed and received 100 pounds of ammonium-phosphate per acre. In this experiment fertilizer was not used at any location. Standard irrigation practices were followed at each location.

To ensure accurate spacing at each location, the experimental areas were seeded by hand. Germination was good. However, where stands were not complete, extra plants thinned from other rows were transplanted to fill out each plot to the required stand. This procedure assured almost perfect stands, although in some cases the transplanted plants failed to produce marketable ears.

Harvesting

The crop at each location was harvested on separate days when visual inspection indicated that approximately 70 per cent of the kernels were in the milk stage. All ears were harvested at one time. Number and weight of marketable ears from each 10-foot plot were recorded.

An attempt was made to record the time necessary to harvest each plot without success. However, the length of time necessary to harvest each row was recorded.

STATISTICAL METHODS

Analysis of Variance

Assuming six treatments, a large number of combinations of plot and block sizes and shapes were worked out. The four block shapes used are illustrated in Figure 1. As much as possible of the entire uniformity data for each location was utilized in each analysis. IBM punched cards were used to estimate the error mean square for each combination of plot and block size and shape. It was assumed that the mean square between plots within blocks estimate was equal to the treatment by block mean square estimate.

Coefficient of Variation

For each plot and block size and shape the coefficient of variation was determined using the formula C.V. = $S/\bar{x}$ (100 per cent) where S is the standard deviation for each plot and blocksize and $\bar{x}$ is the mean yield of the plots used to determine the S value.

Determination of Efficiency

The required number of replications for a given size and shape of plot and block was found by using the formula:

$$\left\{\frac{\text{(C.V. of a single determination)}}{\text{Required S.E. of the mean}}\right\}^2$$

The required S.E. of the mean was arbitrarily set at 3.5 per cent.

Regression Coefficient

In this study plots were formed by combining adjacent units. This tended to give rise to correlation among the units in each plot, making

the variance of plot means larger than if they had been uncorrelated. Smith's empirical law (11) provides a formula for determining the size of the regression coefficient which measures the degree of correlation among adjacent units. $V\bar{x} = V_1/n^b$ is the variance (error mean square) for any size of plot greater than the basic unit, V_1 is the variance of the basic unit, n is the size of the plot, and b is the exponent which measures soil heterogeneity and correlation between adjacent plots.

When values in this formula are transformed to a logarithmic scale the resulting relationship will take the linear regression form, $\text{Log } V\bar{x} = \log V_1 - b \log n$. From this formula $\log V_1 - \log V\bar{x}$ for each plot size of n basic units becomes y and log n becomes x, in $y = bx$ and therefore: $b = \Sigma xy/\Sigma x$.

In general b is between zero and unity. With random selection of the units to make up each plot, b would be unity. As b tends to decrease in size it is an indication of correlation between neighbouring plots, and of greater uniformity of the soil.

Optimum Size of the Plot

The cost per plot may be given by a linear regression $K_1 = K_2 n$, where K_1 is that part of the total cost which is proportional to the number of plots per treatment, and $K_2 n$ is that part of the total cost which is proportional to the total area.

The variance of a treatment mean for plots of n units each is $V\bar{x} = V_1/rn^b$. Transformed to a logarithmic scale this equation becomes

TABLE I.—MEAN COEFFICIENTS OF VARIATION FOR SWEET CORN PLOT AND BLOCK SIZES AND SHAPES

Plot			Mean Coefficients of Variation for all Blocks of Shape*			
Shape		Size				
Length Units	Width Units		A 1x6 Plots	B 2x3 Plots	C 3x2 Plots	D 6x1 Plots
1	1	10′ x 3′	17.4	18.8	17.2	17.8
1	2	10′ x 6′	14.7	14.6	13.2	13.7
1	4	10′ x 12′	14.8	12.0	10.0	10.5
2	1	20′ x 3′	13.0	13.0	13.6	14.4
2	2	20′ x 6′	11.9	11.2	11.1	12.4
2	4	20′ x 12′	14.3	10.8	10.5	10.7
3	1	30′ x 3′	12.6	11.4	13.2	15.3
3	2	30′ x 6′	10.5	9.6	11.4	13.8
3	4	30′ x 12′	13.3	9.6	11.1	12.7
6	1	60′ x 3′	9.1	10.1	12.2	
6	2	60′ x 6′	8.9	7.8	12.2	
6	4	60′ x 12′	12.3	7.6	12.1	
9	1	90′ x 3′	8.4	10.6		
9	2	90′ x 6′	8.6	10.0		
9	4	90′ x 12′	12.2	9.5		

*Block Shapes—*A*, 1 plot long and 6 plots wide; *B*, 2 plots long and 3 plots wide; *C*, 3 plots long and 2 plots wide; and *D*, 6 plots long and 1 plot wide.

$\log V(\bar{x}) = \log V1 - \log r - b \log n^1$. Using the previous expression for cost and the above equation, it can be shown that the cost is minimum when $n = bK_1/(1 - b)K_2$.

This is derived by the use of undetermined multipliers in differential calculus as follows:

Let $\log V(\bar{x}) = L$ and $F = \text{cost} + \lambda (\log V(\bar{x}) - L)$ where λ is any arbitrary value not equal 0. Then $F = K_1r + K_2rn + \lambda (\log V_1 - \log r - b \log n - L)$. Now n and r are to be determined so as to minimize F. Hence $\delta F/\delta n = K_2r\text{-}b\ \tau/n = 0$; $\delta F/\delta r = K_1 + K_2n - \lambda/r = 0$. Solving both equations for λ, $\lambda = K_2rn/b = (K_1 + K_2n)r$. Hence $K_2n = b(K_1 + K_2n)$ and $n = bK_1/(1\text{-}b)K_2$ (10).

RESULTS

Comparison of Plot and Block Sizes and Shapes

Five different lengths and three widths were used for plot shapes in making comparisons with four block shapes. Wherever possible the entire uniformity trial was utilized in the analysis. The coefficients of variation for each plot and block shape were averaged over the three locations as the results from the Lethbridge, Vauxhall, and Cranford sweet corn uniformity trials were quite similar.

Average coefficients of variation for all plot dimensions used in the four block shapes are given in Table 1.

Block shapes of 6 x 1 plots (D) (6 plots long and 1 plot wide) and 3 x 2 plots (C) (3 plots long and 2 plots wide) showed the greatest reduction in variation as plot size increased by increasing plot widths. On the other hand, block shapes of 2 x 3 plots (B) (2 plots long and 3 plots wide) and 1 x 6 plots (A) (1 plot wide and 6 plots long) showed the greatest reduction in variation with increasing plot size as plot length was increased. Thus, in the small sized plots, a great deal of sensitivity is shown to both widening and lengthening of the plots. A closer examination of the coefficients of variation shows a considerable difference due to block shape. Block shapes of 2 x 3 plots (B) seem to be the most effective as far as showing the smallest coefficients of variation as plot size increased.

Thus, these data do not completely agree with most other uniformity trials reported, (1), (2) (3) (9), in which long narrow plots were noted to be more efficient than square-like plots. Rather, the indication of these data is that the long narrow plot may or may not be the most efficient. This was further emphasized when an analysis of variance of the coefficients of variation in Table 1 showed the interaction of plot and block to be significant.

The accuracy of a treatment effect is indicated by the size of its standard error, which is inversely proportional to the square root of the number of replicates. If smaller plots are used with a consequent larger relative standard deviation per plot, more replications will be needed to obtain the same degree of precision.

Using a standard error of a mean of 3.5 per cent, and the averaged coefficients of variation in Table 1, the number of replications required

TABLE 2. THE EFFECT OF SIZE AND SHAPE OF PLOTS AND BLOCKS ON EFFICIENCY OF SWEET CORN EXPERIMENTS

Plot shape			Block size and shape[1]				Block size and shape[1]				Block size and shape[1]			
Length	Width		A	B	C	D	A	B	C	D	A	B	C	D
			1x6 plots	2x3 plots	3x2 plots	6x1 plots	1x6 plots	2x3 plots	3x2 plots	6x1 plots	1x6 plots	2x3 plots	3x2 plots	6x1 plots
Units	Units		Required replication[2]				Required area[3]				Relative cost[4]			
1	1		25	23	24	26	25	23	24	26	25.0	23.0	24.0	26.0
1	2		18	18	14	15	35	36	28	30	23.4	23.4	18.2	19.5
1	4		18	12	8	9	72	48	32	36	14.8	12.8	8.2	17.1
2	1		14	14	15	17	28	28	30	34	18.2	18.2	19.5	22.1
2	2		12	10	10	13	48	40	40	52	22.8	19.0	19.0	24.7
2	4		17	9	9	9	102	72	72	72	42.5	27.9	27.9	37.4
3	1		13	11	14	19	39	33	42	57	20.8	17.6	22.4	40.0
3	2		9	8	11	16	54	48	66	96	22.5	20.0	27.5	55.9
3	4		14	8	10	13	168	96	120	54	67.2	34.4	43.0	
6	1		7	8	12		42	48	72		17.5	20.0	30.0	
6	2		6	5	12		72	60	144		15.8	21.5	51.6	
6	4		12	5			288	120	288		95.8	39.5	94.8	
9	1		6	9			54	81			20.4	30.5		
9	2		6	8			108	144			36.6	48.8		
9	4		12	8			432	288			138.0	92.0		

[1]Block Shapes—*A*, 1 plot long and 6 plots wide; *B*, 2 plots long and 3 plots wide; *C*, 3 plots long and 2 plots wide; and *D*, 6 plots long and 1 plot wide

[2]Replication required to reduce the S.E. of a treatment mean ($\frac{S}{\sqrt{x}}$) to 3.5 per cent

[3]Number of basic 10 x 3 feet units for required replication

[4]Assuming that of the total cost 70 per cent is proportional to the number of replications and 30 per cent proportional to the area

to attain this accuracy are given in columns 5 and 6 of Table 2. As plot size increases the required number of replications decreases. However, as can be seen under the columns headed "Required area", as the number of required replications decreases the area required per treatment increases. If the actual area of land used were a dominant factor in determining plot technique, the smallest size of plot would be the most efficient.

The amount of land occupied by a treatment is, however, not the only item of cost in conducting sweet corn experiments. Actually, land costs make up only part of several items of cost. To determine these costs for the sweet corn uniformity experiments, the length of time and the size of the personnel necessary to carry these experiments to completion was recorded. It was found that the percentage of the total cost proportional to the total area (K_2n) was 59 per cent. The per cent of total cost proportional to the total area will obviously vary from experiment to experiment. The important point is that, whatever the nature of the costs involved in conducting experiments, they should be capable of quantitative estimation and expression.

The relative cost of obtaining the specified accuracy with each plot and block shape can readily be computed as indicated above. From the data in Table 2 relative cost = $rK_1 + nrK_2$ (10) where r is the number of replications per treatment and n is the number of basic units per plot. Assuming 30 per cent of total cost is proportional to the area the relative cost of a plot 1 x 2, in blocks 1 x 6 plots, would be (18) (.70) + (2) (.30) = 23.4. The last four columns of Table 2 give the relative costs on the basis that 30 per cent of the total cost is proportional to the plot area.

Figures 2-5 show the relative cost for the various block shapes when three different assumptions are made about the distribution of the cost. It can be seen from these graphs that, as the per cent of cost due to area increases, the correct selection of plot size becomes very important. This can best be illustrated by selecting two plot dimensions in, say, block shapes of 1 x 6 plots. Thus, plots 2 x 1 and plots 6 x 1 show that, when costs attributable to area amount to only 30 per cent of the total, plots 6 x 1 have a relative cost of 17.5 as compared with 18.2 for plots 2 x 1. If, on the other hand, this cost were 70 per cent instead of 30 per cent then plots 6 x 1 would have a relative cost of 31.5 and plots 2 x 1 a relative cost of 23.9.

It was indicated earlier that block shape affected the relative cost of plots as their dimensions change. It can be seen in Figure 2, and column 12 of Table 2, that block shapes of 2 x 3 plots show the over-all lowest relative costs as plot dimensions change.

The over-all pattern of relative costs shows the small sized plots are on the whole most efficient with sweet corn. As cost due to area decreases, however, the number of basic units could vary from one to three. The shorter and relatively wider plot generally seems more efficient in terms of cost than longer and narrower plots.

Plot and Block Sizes and Shapes Compared Using Smith's Law

Under Statistical Methods, Smith's empirical law was described showing that, when the relationship $V\bar{x} = V_1/n^b$ was transformed to a

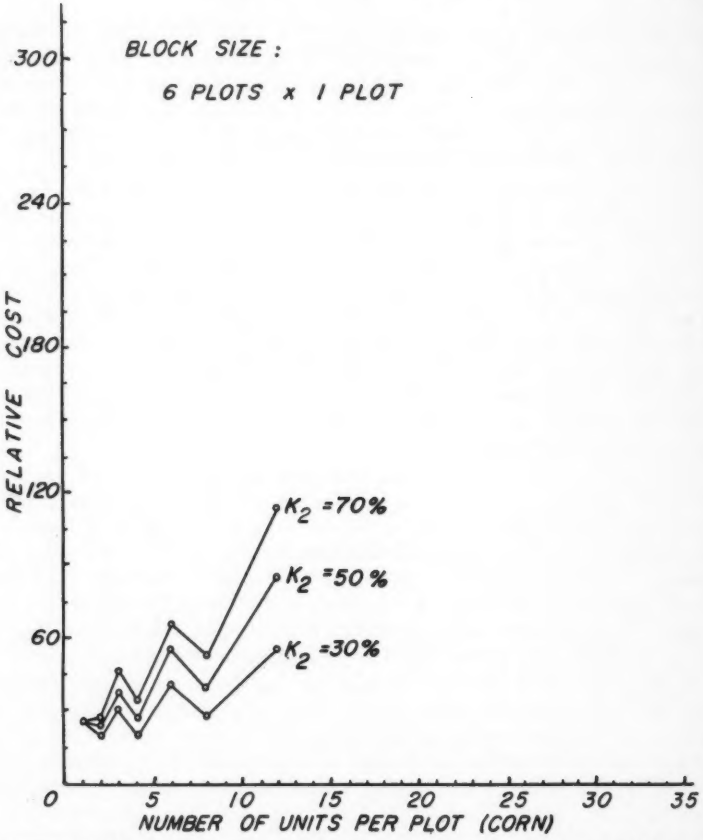

FIGURE 5. The relative cost of obtaining a desired degree of accuracy ($S\bar{x}$ = 3.5 per cent) using different plot sizes and blocks of 6 x 1 plots with sweet corn, assuming various percentages of the total cost to be proportional to the total area used per treatment (K_2).

logarithmic scale, the regression coefficient could be obtained. The averaged regression coefficient for the three locations and four block shapes was found to be 0.3625. The relationship being linear it cannot be used solely for consideration of the reduction in experimental error, or in determining the most efficient plot size. Therefore, cost must be introduced.

Figure 6 gives the optimum plot size for soils that differ in heterogeneity (expressed as b value), and for different assumptions about the percentage of the total cost that is proportional to plot size. In general, Figure 6 agrees with Figures 2-5 regarding the most economical size of plot to use. The curves showing the various cost assumptions emphasize the fact that as costs increase due to area of a plot, the number of basic units that are optimum is sharply reduced until the single basic unit is reached.

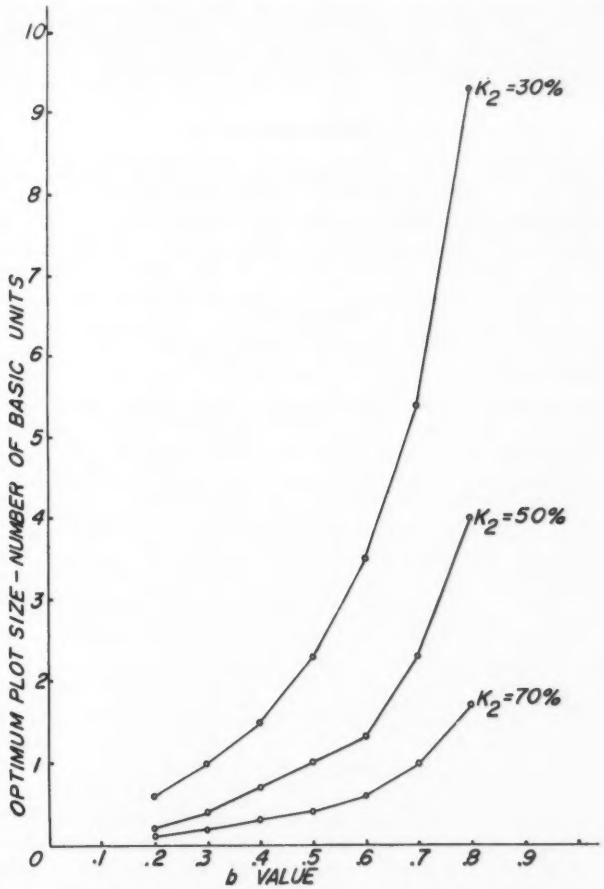

FIGURE 6. The effect on the optimum plot size of soil heterogeneity (b value) and the proportion of the cost of a treatment that is directly related to plot size (K_2).

The regression coefficients for sweet corn ranged from 0.2 to 0.6 which, as indicated in Figure 6, allowed the number of basic units in the optimum plot size to vary from one to three. This is true, providing cost due to total area was only 30 per cent. However, as this cost increases to 70 per cent, optimum size of plot reduces to the basic unit. Thus, using either Smith's relationship or the coefficient of variation method, the most efficient size of plot is the same.

Following through on this interpretation, with the use of the actual K_2n value for the sweet corn experiments estimated to be 59 per cent, the optimum plot size should be very close to the basic unit. Using the

formula $n = bK_1/(1\text{-}b)K_2$ described previously, the optimum size of plot can contain either one or two basic units depending upon the value of b. These results agree with data shown in Figures 2-6.

DISCUSSION

The sweet corn results presented in this study do not fully agree with the results of other uniformity trials with different crops, in which long narrow plots were less variable than short wide plots. The data reported herein indicated that relatively short plots, either one or two rows wide, were most efficient. Furthermore, as plot size increased, there was a tendency for either lengthening or widening of the plots to be equally effective in reducing variation. Possibly these results were due to the use of hybrid sweet corn rather than open-pollinated corn and of highly uniform plot land.

Bryan (2) and Moore and Darroch (9), working with hybrid field corn and hybrid sweet corn respectively, reported similar findings. However, they differed in what they found to be optimum plot size. Bryan stated for hybrid field corn that plots of eight hills distributed in one row were most efficient. Moore and Darroch, working with sweet corn, determined that a single-row 40-foot plot was adequate. Since these workers did not determine the effect of cost on plot size, the results of the experiments reported herein may be closer to the actual requirements of optimum plot size for this crop. A single-row plot, 20 feet in length, was the optimum size of plot, with a plot 10 feet in length and two rows wide almost as efficient. These plot sizes occupied the same land area.

Block shape has a very important influence on plot efficiency. The block shapes of 2 x 3 plots gave the most uniform results and, together with block shapes of 1 x 6 plots, showed that lengthening the plots was more effective in reducing variation than widening the plots. On the other hand, block shapes of 6 x 1 plots and block shapes of 3 x 2 plots showed that widening the plots was more effective than lengthening the plots in reducing variation (see Table 1). This emphasizes the importance of plot and block shape and arrangement when designing sweet corn experiments. Not only must soil variability be considered, but also the manner in which block shape will affect plot shape efficiency.

The optimum plot size of one or two basic units, depending upon the cost assumptions, has an important bearing on quality studies when testing treatments or varieties. Cut kernels from the ears of each unit are used as a measure of quality. It was noted in the "Materials and Methods" section that the quantity of kernels cut from the ears of the basic unit was adequate for further testing of quality. Immer (5) pointed out that what was necessarily an optimum plot size for yield need not be optimum or adequate for quality studies. For sweet corn it would seem that these factors can be studied together using the optimum size of plot for yield.

It should be noted that the particular sweet corn hybrid used for these studies exhibited only slight plot to plot variation. Since this was a typical commercial hybrid there is reason to conclude that this uniformity

of performance may hold for other sweet corn hybrids. Despite soil differences, the results were comparatively constant from location to location, giving support to the belief that the optimum size of plot may have broad application.

ACKNOWLEDGEMENT

The author gratefully acknowledges the contribution of W. A. Frazier, Horticulturist, and L. D. Calvin, Statistician, Oregon State College, Corvallis, Oregon, for their keen interest, guidance and encouragement given throughout the course of this study and in the preparation of the manuscript.

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INFLUENCE OF THE ESTER OF 2,4-D APPLIED AT VARIOUS GROWTH STAGES FOR THE CONTROL OF RUSSIAN THISTLE (*SALSOLA PESTIFER* NELS.) IN FLAX[1]

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ABSTRACT

Linseed flax, growing with naturally occurring infestations of Russian thistle at Lethbridge, Alta., was treated with three rates (2, 3, and 6 oz. acid equivalent per acre) of the butyl ester of 2,4-D at 5-day intervals from the seedling stage to boll formation in each of the 5 years 1952 to 1956, inclusive.

Two peaks of weed control were determined, the first being at the early growth stages between 19 and 35 days after crop seeding when some measure of weed stand reduction was obtained. The second peak occurred from 45 to 60 days after seeding when the Russian thistle plants were 6 to 8 in. tall, the control being characterized by a "curl-down" of weed growth which persisted to harvest time. Mature flax height was relatively unaffected except when the crop was treated at or near the first-bud stage of growth. Height of flax above the weed mass was 9.7 and 13.2 in. in 1954 and 1955, respectively, as a result of the best-timed 6-oz. treatment. Such height differences would make it possible to combine-harvest flax seed containing a minimum of weed material. Maturity delays of only 2 or 3 days resulted from treatments made within 1 month after seeding, whereas delays of up to 33 days resulted from treatments made when the flax was in full bloom. On the average for all three treatment rates, flax yields equal to or better than those from untreated weedy plots were obtained in each of the 5 test years when the 2,4-D was applied prior to 36 days after seeding. Thus, for adequate control of a Russian thistle infestation and the undelayed harvest of a good yield of flax seed, it would appear that, 25 to 30 days after seeding, the crop should be treated with a 6-oz. rate of the butyl ester of 2,4-D. Slightly earlier treatment is indicated if cool, wet conditions prevail after seeding.

INTRODUCTION

The introduction of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) has provided useful materials for weed control in flax, a crop that does not compete well with weeds. The effects of 2,4-D and MCPA on flax have been studied (1, 7, 10, 11, 12, 13), and the least susceptible growth stages for treatment were found to be from late seedling (flax 1-1/2 to 2 inches tall) through to pre-bud stage. Flax has been found better able to tolerate MCPA than 2,4-D at equal rates (1, 8, 9, 12). Removal of weed competition to flax, either by hand-weeding (3, 16) or by the use of herbicides (2, 3, 18), resulted in higher crop yields, but relatively early treatment seemed to be essential (18, 20).

The greatest germination of Russian thistle has been found to occur in the early spring (4, 21) and from relatively shallow depths (5). However, Chepil (4) found that germination continued well into June at Swift Current, Saskatchewan, although to a diminished degree. Similar observations have been made by the author at Lethbridge, Alberta. Russian thistle has been classed as intermediate in susceptibility to 2,4-D and as resistant to MCPA (14). The weed can be killed by moderate rates of 2,4-D in the early seedling stages but becomes fairly resistant to the herbicide as

[1] Contribution from Soils Section

soon as it reaches a height of 2 inches and begins to branch. Work at Lethbridge indicates the superiority of the ester of 2,4-D over either sodium or amine salts for the control of Russian thistle (17, 18) and the comparative resistance of the weed to MCPA (19).

Because Russian thistle seeds germinate over a relatively extended period in the spring and because the seedlings rapidly become resistant to 2,4-D, it is important to determine the time of treatment at which optimum results can be obtained in a weakly competitive crop such as flax. The tests reported in this paper were begun in 1952 to determine the relative value of three rates of the butyl ester of 2,4-D applied to flax, at intervals from the seedling stage through to boll formation, for the control of Russian thistle.

MATERIALS AND METHODS

Tests were conducted on a dry-land silty clay loam at Lethbridge, Alberta, in each of the 5 years from 1952 to 1956. Linseed flax (var. Dakota, 1952 and 1953; var. Rocket, 1954; and var. Redwood, 1955 and 1956) was seeded on fallow using a press drill. With the exception of 1952, when the flax was seeded on April 26, all seeding was done during the third week of May, which is within a few days of the normal seeding time for flax in the Lethbridge area. Eight- by twenty-foot plots were established in the seeded area, which contained a naturally occurring infestation of mixed annual weeds, with Russian thistle comprising 95 per cent or more of the weed stand. The infestation varied from year to year, ranging from very light in 1956 to medium heavy in 1954.

Sufficient area was seeded in each season to allow for the treatment of quadruplicate plots with the butyl ester of 2,4-D, at rates of 0, 2, 3, and 6 ounces acid equivalent per acre, at 12 growth stages. Beginning when flax was from 1/2 to 2 inches tall, treatments were applied at 5-day intervals, or as close to 5-day intervals as the weather would allow, until 10 sets of treatments had been applied in 1952 and 1953 and 12 sets in the other 3 years. At the time of the last treatment the flax was up to mature height and in full-boll formation. A split-plot design was used, with the growth-stage blocks randomized within each of the four replicates and the application rates randomized within each growth-stage block.

All treatments were applied with a hand-propelled, compressed-air boom sprayer. The solution rate varied from year to year between 11.7 and 13.4 gallons per acre, and water alone was applied to all zero-rate check plots.

A visual estimate of control of Russian thistle was made at harvest time in each year, using a rating score from 0 to 10 to indicate a range from no visible effect of treatment to complete kill of the weeds. Because of the variability of weed stand when the infestation was light (1956), and because of the dense ground cover when the infestation was heavy (1954), it was impossible at harvest time to assess the weed kills resulting from the early treatments. Height measurements of mature flax and Russian thistle were taken at six locations in each plot in 1954 and 1955. Accurate height measurement of the light infestation of Russian thistle in 1956 was diffi-

TABLE 1.—GROWTH STAGES OF FLAX AND RUSSIAN THISTLE RECORDED AT TWELVE TREATMENT DATES AT LETHBRIDGE, ALBERTA, IN 1954

Date of application	Days after seeding	Growth stage of flax	Growth stage of Russian thistle
June 3	16	Cotyledon to 2 or, at most, 4 small leaves, shoot above cotyledons to 1/2" long	Variable, mostly early "pine-leaf" with very small first leaves
June 8	21	Variable, cotyledon to 1" shoot, mostly with short shoot and 2 to 4 small leaves	Variable, mostly early to mid "pine-leaf'
June 14	27	Variable, early shooting to 2" shoot, most with 1-1/2" shoot, 6 leaves unfolded	Variable, early cotyledon to full "pine-leaf", beginning to branch, 3" tall
June 19	32	Variable from 1-1/2" tall with 4 expanded leaves to 4" tall with 20 leaves, average height approximately 2-1/2"	Variable from early "pine-leaf" to 3" tall with 2 branches 1-1/2" long, average 2" tall with first small branches
June 23	36	Variable, 2" to 5-1/2" tall with from 6 to 26 leaves, average height approximately 3-1/2"	Variable, "pine-leaf" to 4" tall with up to 6 branches, longest of which were 3", average 2-1/2" tall and 2 to 4 branches
June 29	42	Variable, 3" to 9" tall with from 14 to 30 or more leaves, average height approximately 6"	Variable, "pine-leaf" to 6" to 7" tall with 6 branches to 5" length, average 3" to 4" tall
July 3	46	Variable, 6" to 12" tall, tallest with buds enclosed in top leaf cluster, average 9" tall in pre-bud stage	Variable, mid "pine-leaf" to 6" to 8" tall, average 4" tall with lowest branches 2-1/2" long; progressively fewer seedlings
July 8	51	Variable, 12" to 15-1/2" tall in bud to early boll, average 14" in bud to first bloom	Main crop from 6" to 11" tall with lower branches from 4-1/2" to 9-1/2" long, some spindly unbranched plants to 3"
July 13	56	2/3 bloom, 13" to 15-1/2" tall from first bloom to half-formed bolls, average 14" to 15" in bloom to early boll	6-1/2" to 11-1/2" tall, 6-1/2" plants mostly spindly with few short branches, 11-1/2" plants well branched and bushy
July 18	61	15" to 17" tall, late bloom to boll, most bolls full size	Mostly to 15" tall, well branched and bushy, pre-bloom
July 23	66	Flowering mostly completed, bolls 1/4 to full size, mostly full size	As above, blooming
July 28	71	15" to 17" tall, full-sized bolls, well formed seed	As above and fully branched, late bloom

cult and, therefore, data are not available. In the 3 years, 1953, 1955, and 1956, weather conditions allowed the taking of yield samples at the same relative stage of ripeness, and in these years the harvest dates were used to obtain maturity data. In each of the 5 test years yield samples were obtained by hand-pulling the flax from four rod-length rows in each plot.

The variance method of analysis, applicable to a split-plot test as described by Paterson (15), was used to analyse all data. The square root transformation, as outlined by Cochran (6), was applied to the weed-control rating data.

RESULTS AND DISCUSSION

The growth stages of Russian thistle and flax were not identical in all years at the different treatment dates. However, to illustrate the growth stages as related to treatment dates, the information taken in 1954 is presented in Table 1. In 1954 flax reached the bud to first-bloom stage 51 days after seeding. The time required to reach this same growth stage in the other test years ranged from 50 to 56 days.

The harvest-time control ratings of Russian thistle obtained for the three rates of 2,4-D application in each of the 5 years are shown graphically

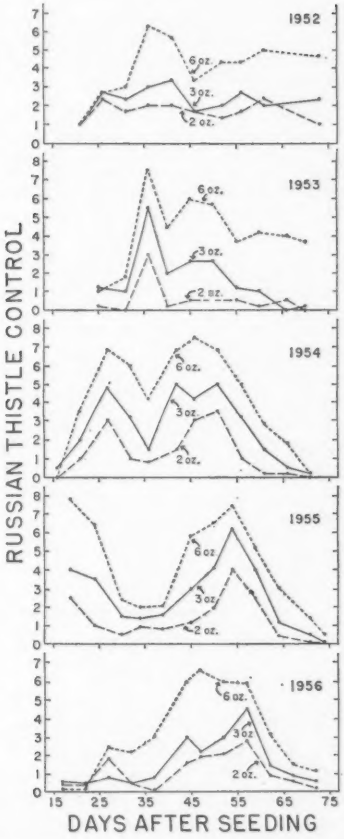

FIGURE 1. Harvest-time control rating for Russian thistle as affected by the application of three rates of the butyl ester of 2,4-D at various times after flax seeding. Lethbridge, Alberta, 1952–1956.

in Figure 1. Two peaks of control are evident. The first peak resulted from the effect of the herbicide on young Russian thistle plants from 19 to 35 days after crop seeding. The location of this peak of control was affected by the date of germination of Russian thistle seeds, the germination at all times being from shallow depths and dependent upon the presence of adequate surface moisture resulting from rains. In 1955 the greatest control occurred at the earliest treatment date, presumably because cool and excessively moist conditions prevailed immediately after seeding, which allowed for early and uniform establishment of the Russian thistle infestation.

The second peak of weed control was obtained from 45 to 60 days after seeding when the Russian thistle plants were well advanced and in the pre-flowering stages, being from 6 to 8 inches tall with from 6 to 10 well-formed branches up to 5 to 7 inches in length. The control obtained at this stage consisted of a "curl-down" of the main stem and branches to form a mat of growth close to the ground, which condition persisted to time of harvest.

FIGURE 2. Height of flax as affected by the application of three rates of the butyl ester of 2,4-D at various times after seeding. Lethbridge, Alberta, 1954, 1955.

FIGURE 3. Height difference of flax above Russian thistle as affected by the application of three rates of the butyl ester of 2,4-D at various times after seeding. Lethbridge, Alberta, 1954, 1955.

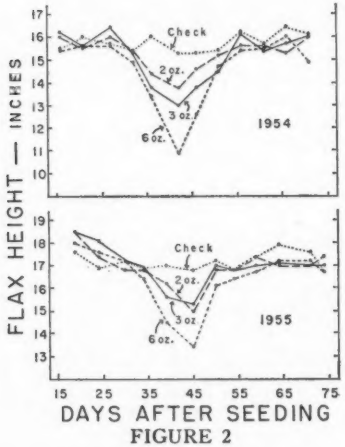

FIGURE 2

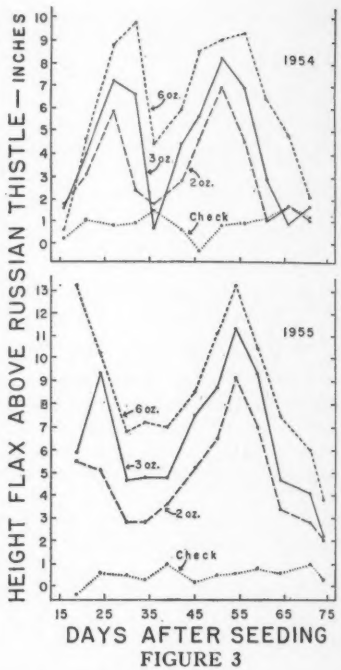

FIGURE 3

The most effective control was obtained at the early stages of Russian thistle growth, this being evident in the years 1952, 1953, and 1955, when it was possible to assess at harvest time the weed kills and growth suppression resulting from the early treatments. The "curl-down" resulting from the application of the ester of 2,4-D at the later growth stages was of value for keeping a large bulk of the weed growth out of the upper portion of the flax crop. However, by the time these treatments were made the major competitive effects of the weeds had occurred.

The 2,4-D treatments applied from 35 to 50 days after seeding caused a reduction in flax height, as shown in Figure 2. The height reduction increased with increasing rates of 2,4-D and was greatest when the flax was at or near the first-bud stage of growth. The maximum reduction of slightly over 4 inches recorded in 1954 and 1955 was caused by the 6-ounce applications.

From the standpoint of ease of harvest, the actual height reduction of the flax would not be as important as would the height difference between the flax crop and the weed mass remaining after 2,4-D treatment. Figure 3 gives a graphic presentation of the height differences in the two seasons in which measurements were taken. The untreated Russian thistle growth tended to be equal in height to the mature flax crop. The height differences between the flax and the Russian thistle of up to 9.7 inches in 1954 and 13.2 inches in 1955, resulting from the 6-ounce treatment with the

FIGURE 4. Maturity delay of flax resulting from the applicaion of three rates of the butyl ester of 2,4-D at various times after seeding. Lethbridge, Alberta, 1953, 1955, 1956.

FIGURE 5. Yield of flax as affected by the application of three rates of the butyl ester of 2,4-D at various times after seeding. Lethbridge, Alberta, 1952–1956.

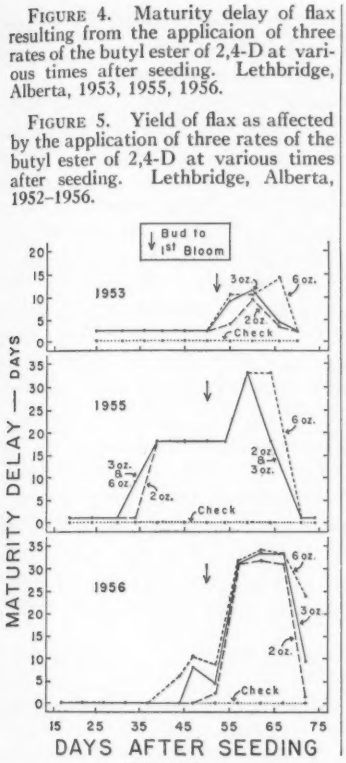

FIGURE 4

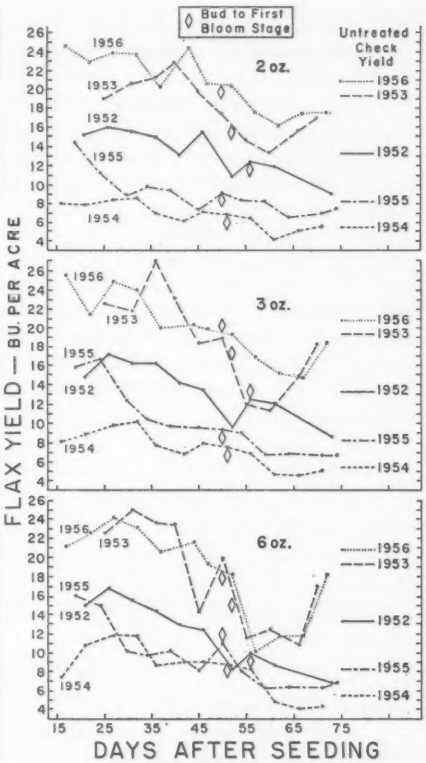

FIGURE 5

ester of 2,4-D, would be sufficient to allow for the recovery of a maximum amount of flax seed with the minimum amount of weed material by combine harvesting. These height differences were obtained from treatments made not later than 30 days after seeding. Even though almost equal height differences resulted from later treatments, such treatments would be of questionable value in the light of evidence yet to be presented. The trends shown in the graphs of Figure 3 follow closely those of the weed-control rating data in Figure 1, indicating that, to a large degree, the control ratings were based on the height of the Russian thistles at harvest time.

Visual observations showed that greater injury to the flax crop resulted from increasing rates of 2,4-D and with advancing age of the flax from seedling through to bloom and early-boll formation. The kinds of injury noted included stem bending and shortening, fusion of leaves immediately below the flowering branches, boll fusion and abortion, and maturity delay. Information on maturity delay was obtained in 3 of the 5 test years, and the results are shown in Figure 4. The 2,4-D treatments made in the

early stages of flax growth, up to 30 days after seeding, caused only minor delays in maturity, whereas treatments applied from the late vegetative stage through the flowering stages caused very serious delays.

Flax seed yield data are presented in Figure 5 for each of the three rates of 2,4-D application in all 5 years of testing. It was found that, on the average for all three rates of 2,4-D application, yields equal to or better than untreated check yields were obtained in 1956 from treatments made prior to 36 days after seeding, this being the year in which the point of equal yield was reached at the earliest time after seeding. On the average for the 5 years, yields equal to or better than check yields were obtained from the 6-ounce rate of 2,4-D applied up to 44 days after seeding. The data show that good flax yields can be expected if the ester of 2,4-D is applied at 6 ounces per acre prior to 35 days after crop seeding, even under conditions of a light weed infestation. In general, significant yield increases were obtained when treatments were made between 25 and 35 days after seeding, at which time the flax ranged between 1-1/2 and 5 inches in height.

The tests indicate that, to control Russian thistle and to obtain the maximum height of flax above the weed mass, normal or near-normal maturity, and good crop yield, linseed flax grown at Lethbridge, Alberta, can be treated with 6 ounces acid equivalent per acre of the butyl ester of 2,4-D between 25 and 30 days after flax seeding. However, the results obtained in 1955 indicate that under cool and wet conditions most beneficial results may be obtained from a treatment applied a few days earlier.

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A STUDY OF THE MEGAGAMETOPHYTE IN *PRUNUS AVIUM* AND ITS RELATION TO FRUIT SETTING[1]

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ABSTRACT

A cytological study of the megagametophyte in *Prunus avium* was undertaken to determine to what extent delayed development, cytological irregularities, or degeneration might account for failure of this fruit to set commercial crops. In 1957, under high temperatures, the proportion of embryo sacs with functional egg cells decreased rapidly following anthesis. In 1958, under low temperatures, the maximum proportion of functional embryo sacs at any one time was much lower than in 1957, and the proportion of embryo sacs with functional egg cells began to decrease within 2 days of anthesis. At anthesis in both years sufficient functional embryo sacs were present to allow a commercial set of fruit. The importance of cross-pollination immediately at anthesis is emphasized. The conclusions are supported by studies of pistils hand-pollinated at intervals after anthesis.

INTRODUCTION AND LITERATURE REVIEW

Sweet cherries in Ontario frequently fail to set a commercial crop of fruit even with provision for cross-pollination and in the absence of damaging spring frosts, although the latter have reduced crops in some instances.

Studies by Dorsey (2), Hartman and Howlett (3), and Howlett (4) showed that delayed development of the embryo sac, and embryo sac disintegration following anthesis, can explain failure of fruit to set in certain apple varieties. Similarly, in a study of seedlessness in grapes, Stout (9) found a large proportion of abortive embryo sacs.

In *Prunus avium*, Tulasne (11) and Went (13) studied the form of the embryo sac, and Péchoutre (7) and Ruehle (8) described its development; but no attempt was made to relate these studies to the commercial production of cherries. Tukey (10), in an investigation of the causes of embryo abortion in early ripening sweet cherries, observed no delayed development, cytological irregularities, or degeneration of the embryo sac. Bradbury (1), on the other hand, in studying differences between developing and aborting sour cherries (*P. cerasus*), described degeneration in gametophytes of young functional ovules. The emphasis in Tukey's and Bradbury's work was on stages following petal fall so that no information is available on the proportion of embryo sacs that are mature and functional during the period between anthesis and petal fall.

The primary objectives of the present study were to obtain quantitative as well as qualitative information with respect to the female gametophyte in sweet cherry, to relate this information if possible to fertilization and fruit setting behaviour, and to determine to what extent delayed development, irregularities, or degeneration of the embryo sac might be responsible for the irregular and unsatisfactory fruit-set in this crop.

[1]Part of a dissertation presented to the Graduate School of The Ohio State University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

MATERIALS AND METHODS

Sampling Procedure, Columbus, Ohio, 1957

Four Windsor sweet cherry trees in the orchard of the Department of Horticulture and Forestry at The Ohio State University were used as a source of material. Trees 107 and 108 were planted in 1950, and trees 17 and 18 were planted in 1948. Protection from cross-pollination was provided by covering several limbs on each of the selected trees with cheesecloth bags before any of the flowers had reached anthesis. Flowers reaching anthesis on April 21 and April 23 were collected from each of the four trees at anthesis and at 2-day intervals thereafter. The last collections were made 6 days after anthesis; each collection consisted of approximately 18 pistils. Temperature and stage-of-development records are presented in Table 1.

Sampling Procedure, Vineland Station, Ontario, 1958

Samples were collected from three Windsor trees and one Vista tree in orchards of the Horticultural Experiment Station, Vineland Station. The three Windsor trees were planted about 1933. The Vista tree was planted in 1951.

On the Windsor trees protective cheesecloth bags were placed on several limbs. The first flowers to reach anthesis did so on April 28. Separate collections of unpollinated pistils were made from certain limbs from zero to 8 days after anthesis.

Approximately 100 Windsor flowers which reached anthesis on April 29 and 30 were hand-pollinated and collected at intervals after anthesis.

Temperature and stage-of-development records are presented in Table 2.

TABLE 1.—MAXIMUM AND MINIMUM TEMPERATURES AND STAGE-OF-DEVELOPMENT IN THE ORCHARD PRECEDING AND DURING THE SAMPLING PERIOD, COLUMBUS, 1957

Date	Temperature*		Stage-of-Development
	Min.	Max.	
April 16	42	52	Bud scales expanded, tips of sepals exposed
17	45	51	
18	46	52	
19	44	66	A few blossoms at white-tip stage
20	52	80	Many blossoms in "balloon" stage
21	59	82	A few flowers at anthesis
22	57	79	
23	57	75	Trees in full bloom
24	59	80	
25	60	85	Petals off most flowers
26	60	82	
27	61	83	
28	56	84	
29	53	74	
30	48	75	

*Maximum and minimum temperatures are those recorded on a hygrothermograph during the 24-hour period preceding 8 a.m. on the given date.

TABLE 2.—MAXIMUM AND MINIMUM TEMPERATURES AND STAGE-OF-DEVELOPMENT IN THE ORCHARD UP TO AND INCLUDING MOST OF THE SAMPLING PERIOD, VINELAND STATION, 1958

Date	Temperature*		Stage-of-Development
	Min.	Max.	
April 19	36.0	63.5	Bud scales expanded, sepals exposed
20	42.0	75.0	
21	46.0	61.5	Bracts expanded, blossoms separate
22	42.5	55.0	Sepals beginning to separate
23	39.0	63.5	
24	46.0	80.0	Tips of most petals exposed
25	38.0	52.0	
26	33.5	52.5	A few blossoms in "balloon" stage
27	34.5	51.5	
28	43.5	49.5	Many blossoms in "balloon" stage
29	42.5	55.5	A few flowers at anthesis
30	32.0	56.0	More flowers at anthesis
May 1	36.5	62.0	Trees in full bloom
2	36.5	46.0	
3	41.0	65.5	
4	44.5	51.0	
5	36.0	46.5	
6	32.5	50.5	Early opening flowers slightly wilted
7	42.5	56.0	Flowers slightly wilted, anthers dry and dark brown
8	38.5	69.0	First weather favourable to bee flight, petals falling from early opening flowers
9	39.0	69.2	
10	32.0	69.5	All early opening flowers past petal fall
11	40.5	78.5	
12	39.5	64.4	Petals off most flowers
13	39.5	67.4	

*Maximum and minimum temperature readings were made between 8 and 9 a.m. for the preceding 24-hour period.

Since the period in which flowers reached anthesis was prolonged (Table 2) and only a few flowers reached anthesis at any one time, it was impossible to work with very large numbers of hand-pollinated Windsor flowers. Accordingly a similar experiment was undertaken with Vista variety which bloomed several days after Windsor. Hedelfingen pollen, collected a day or so in advance and ripened overnight at room temperature, was stored at 40° F. until required for use. Hedelfingen pollen was used in pollinations of both Windsor and Vista varieties.

Cytological Methods

The methods used in preparing material for microscopic examination were essentially the same in 1957 and 1958. Material was fixed within half an hour of collecting in Belling's Modified Novashin fluid described by Johansen (5). The vials containing the samples were unstoppered and placed in a dessicator jar under vacuum for 20 to 24 hours to hasten penetration by the fixative. Materials were stored in the fluid until it was convenient to proceed. Pistils were washed 24 hours and dehydrated according to the tertiary butyl alcohol method of Johansen (5). Pistils were embedded in The Ohio State University Department of Anatomy "summer formula" embedding mixture and cut serially at 10 to 14 microns

PLATE I

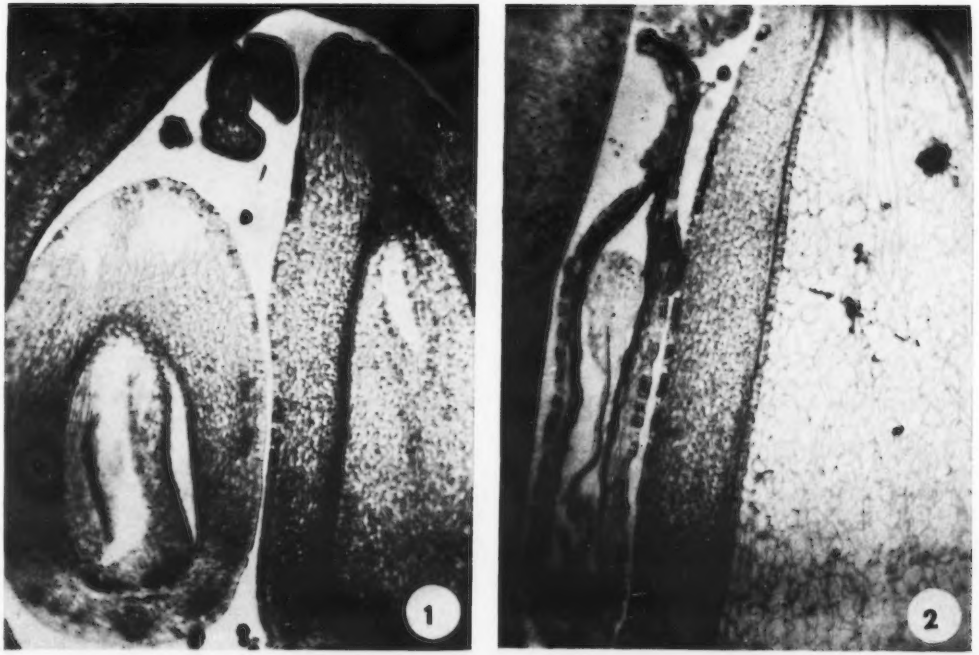

FIGURE 1. Large primary ovule, *right*, and secondary ovule, *left*, in pistil collected at anthesis, 1957. (x 110).

FIGURE 2. Large primary ovule, *right*, and secondary ovule, *left*, pistil collected 4 days after anthesis, 1957. (x 110).

PLATE II

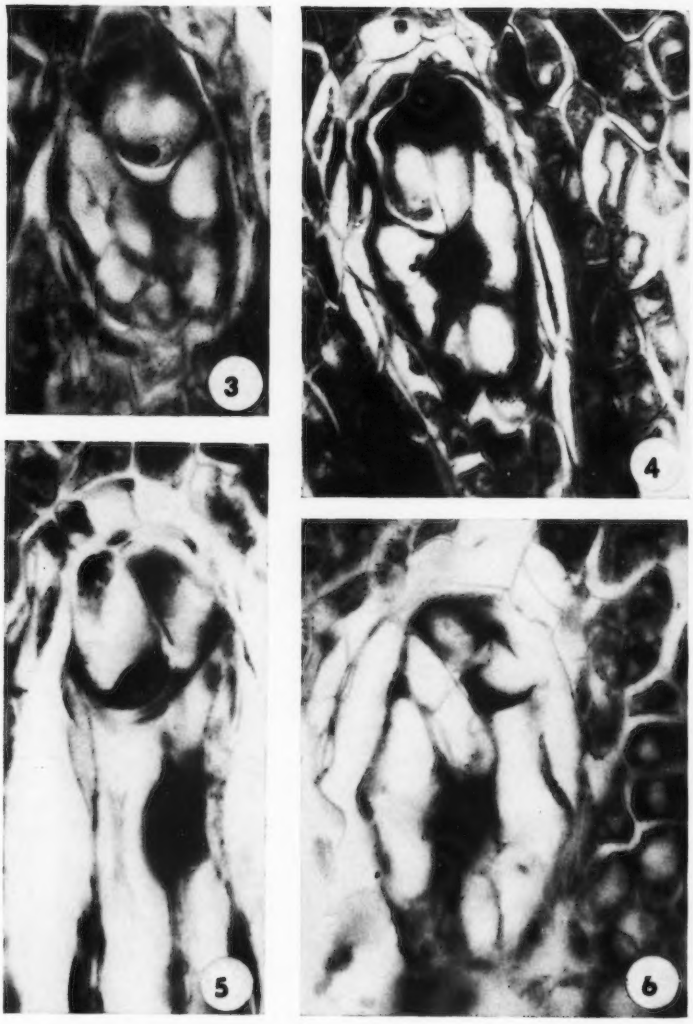

FIGURE 3. Functional egg cell at anthesis, 1957. (x 875).

FIGURE 4. Polar nuclei about to fuse at anthesis, 1957. The synergids are not quite in focus. The functional egg cell of this embryo sac was found in the adjacent section. (x 875).

FIGURE 5. Enlarged embryo sac with functional egg cell and fusion nucleus, the fusion nucleus is out of focus, at anthesis, 1957. (x 875).

FIGURE 6. Embryo sac with degenerate egg cell 2 days after anthesis, 1957. The fusion nucleus is out of focus. (x 875).

thickness. In 1957 the ribbons were affixed to the slides with Haupt's gelatin adhesive (5). It was found in 1958 that a considerable saving in time could be realized by using the adhesive described by Venning (12), prepared from egg white, glycerine, sodium salicylate, and distilled water. Especially in 1958 difficulty was encountered due to loss of sections from the slides during the steps in staining. These losses were minimized by washing the slides in a laboratory detergent or cleaning compound, rinsing in distilled water, and wiping dry with cheesecloth or a lint-free absorbent paper wipe. Sections were mordanted in iron alum, stained in Haidenhain's haematoxylin according to a schedule similar to that described by Johansen (5), and mounted in Technicon mounting medium. Sections were examined with a Leitz research microscope equipped with a 1.32 N.A. apochromatic oil immersion objective (90x) and periplan oculars. All photomicrographs were prepared with a Leitz "MAKAM" camera.

RESULTS

Primary and Secondary Ovules

It was usually possible in pistils collected at anthesis, as well as later, to distinguish between the primary or functional ovule and the secondary or non-functional ovule. In the primary ovule comparable tissues had a greater affinity for haematoxylin stain than the secondary ovule. The secondary ovule was usually constricted and narrow, and its nucellus drawn away from the integuments. The micropylar region of the nucellus in secondary ovules was frequently almost spherical (Figure 1) and contained a small, round, embryo sac in various stages of degeneration. Occasionally the only difference between primary and secondary ovules was that the former had a long tapering calotte while that of the latter was short and blunt. Figure 1 illustrates the marked difference frequently noted between the two ovules at anthesis. Figure 2 shows the even greater difference which was common in collections made several days after anthesis.

Fertilization in only one ovule is required for fruit set since one ovule, the secondary, usually aborts and only one seed is usually present in the pit of a mature fruit. Since the object of the present study was to discover any conditions of the embryo sac which might account for differences in fruit set, critical attention was given only to embryo sacs present in primary ovules.

Unpollinated Windsor Flowers, 1957

There were very marked differences between collections made at 2-day intervals in 1957. The data in Table 3 show that at anthesis almost all embryo sacs had reached the 8-nucleate stage and almost two-thirds of the embryo sacs had differentiated an egg cell. There was one 4-nucleate embryo sac and only one embryo sac was noted with a degenerate egg cell. Antipodals were usually degenerated in flowers collected at anthesis, but their traces could be recognized in almost every mature embryo sac. Figures 3, 4 and 5 are typical of embryo sacs collected at anthesis in 1957.

In flowers collected 2 or more days after anthesis no embryo sac was found which had not reached the 8-nucleate stage, and in all but

TABLE 3.—CONDITION OF EMBRYO SACS IN PRIMARY OVULES OF UNPOLLINATED WINDSOR SWEET CHERRY FLOWERS, COLUMBUS, 1957

	Days after anthesis			
	0	2	4	6
	Percentage of embryo sacs in various stages of development			
Megaspore mother cell to 4-nucleate embryo sac	4.8	—	—	—
8-nucleate embryo sacs				
Egg cell not differentiated	28.6	3.7	—	—
Egg cell differentiated	61.9	44.4	20.0	—
Embryo sac degenerated or irregular	4.8	51.9	80.0	100.0
	Number of embryo sacs examined			
	21	27	25	9

one embryo sac an egg cell had been differentiated. The polar nuclei had fused in all embryo sacs examined. The most striking difference between the flowers at anthesis and 2 days later was the great increase in the proportion of egg cells degenerating in the latter (Table 3). Over half of the embryo sacs collected 2 days after anthesis had degenerate egg cells, an example of which may be seen in Figure 6. In degenerate egg cells the nucleus or nucleolus could not be differentiated and the cytoplasm had so much affinity for haematoxylin stain that the entire cell could be recognized only as a black strand of material in the micropylar end of the embryo sac. Of the remaining embryo sacs having a functional-appearing egg cell, many had at least one synergid with signs of degeneration—a condition not noted at anthesis.

The data in Table 3 show that 4 days after anthesis 80 per cent of the embryo sacs either had degenerate egg cells or exhibited some other irregularity which rendered them non-functional. Such an irregularity is illustrated in Figure 7 where both ovules were degenerate and had the appearance of secondary ovules.

Six days after anthesis no functional egg cells were found, although at this time the fusion nucleus usually appeared functional. Rapid elongation of the embryo sac took place during the 6-day period following anthesis in which pistils were collected.

Unpollinated Windsor Flowers, 1958

The samples of unpollinated flowers collected as described in 1958 were examined separately. However, since there was considerable variability in stages present in pistils collected at any one time, and partly because the loss of sections in the staining schedule reduced the useful number of pistils in each collection, it was not satisfactory to group the data on the basis of collections at daily intervals following anthesis. Marked differences between groups collected in 1958 were noted, however, when the data are presented according to 2-day intervals. These data are presented in Table 4.

PLATE IIIA

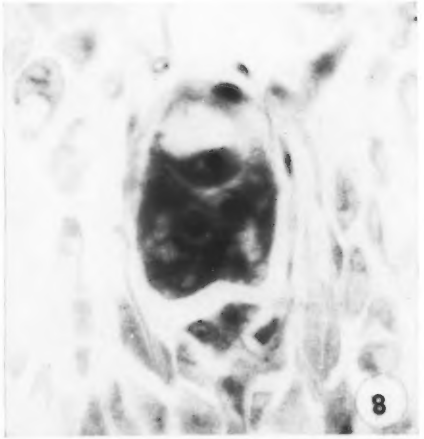

FIGURE 7. Pistil containing two degenerate ovules collected 4 days after anthesis, 1957. (x 110).

FIGURE 8. Embryo sac containing apparently functional egg cell described as Type A, polar nuclei and antipodals. Collected 1 day after anthesis, 1958. (x 875).

PLATE IIIB

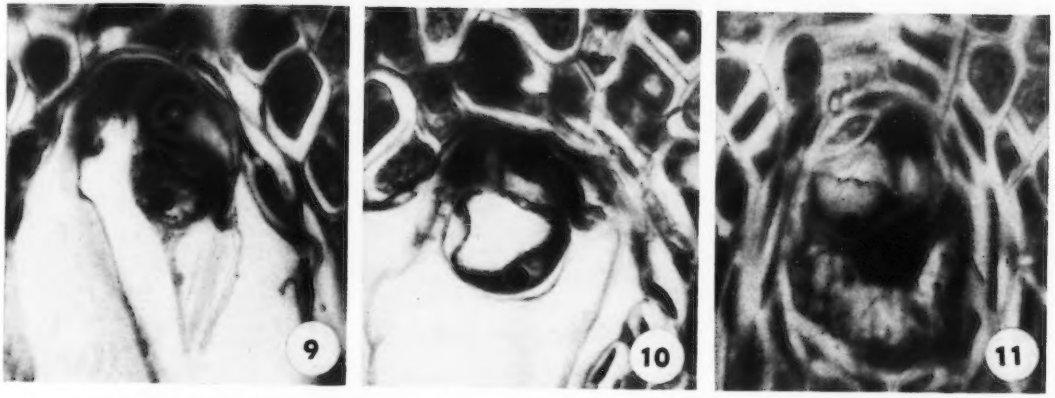

FIGURE 9. Egg cell described as Type B from an unpollinated flower collected 8 days after anthesis, 1958. (x 875).

FIGURE 10. Egg cell described as Type C from an unpollinated flower collected 8 days after anthesis, 1958. (x 875).

FIGURE 11. Embryo sac containing an egg cell described as Type D collected 1 day after anthesis, 1958. (x 875).

TABLE 4.—CONDITION OF EMBRYO SACS IN PRIMARY OVULES OF UNPOLLINATED WINDSOR SWEET CHERRY FLOWERS, VINELAND STATION, 1958

	Days after anthesis				
	0	2	4	6	8
	Percentage of embryo sacs in various stages of development				
Megaspore mother cell to 4-nucleate embryo sac	16.0	10.5	2.2	3.1	0
8-nucleate embryo sac					
Egg cell not differentiated	52.0	32.6	20.0	12.5	4.8
Egg cell differentiated					
Egg cell type* A	12.0	29.1	17.8	6.3	11.1
B	0	1.2	4.4	9.4	15.9
C	0	8.1	4.4	21.9	30.2
D	0	8.1	31.1	25.0	11.1
Embryo sac too irregular to classify	20.0	10.5	20.0	21.9	27.0
	Number of embryo sacs examined				
	25	86	45	32	63

*The type of egg cells, A, B, C, and D are described in the text.

The majority of embryo sacs collected at anthesis in 1958 (Table 4) were not nearly as advanced as those collected at anthesis in 1957 (Table 3). At anthesis in 1958 an egg cell could be recognized in only 12 per cent of the embryo sacs examined, although 64 per cent of the embryo sacs had reached the 8-nucleate stage. Only a small proportion of the embryo sacs examined in 1958 were 4-nucleate or at an earlier stage of development. Sufficient early stages of development were observed, however, to support the findings of previous workers that the embryo sac is monosporic and 8-nucleate and develops according to the *Polygonum* type described by Maheshwari (6). Any megaspore mother cells observed at anthesis were quite small and did not stain as darkly as might have been expected. One ovule was observed with an almost linear tetrad of megaspores.

In collections after anthesis in 1958 the appearance of mature egg cells was more variable than in 1957. In 1958 it was not always possible to recognize egg cells as being either clearly functional or clearly degenerate. After some study it became apparent that egg cells might conveniently be described as Types A, B, C, and D (Figures 8,9,10,11).

Type A included any egg cell which had a clearly defined cell wall, nucleus, and nucleolus. The cytoplasm of the egg cell had some affinity for haematoxylin but did not stain darkly. The surrounding cytoplasm of the embryo sac stained similarly. The nucleolus stained very darkly, and the remainder of the nucleus slightly if at all. There seemed no doubt on the basis of appearance that Type A was functional. Type A was essentially the same as those egg cells considered functional in 1957.

Type B included egg cells in which the cytoplasm had a reticulate appearance and stained very lightly. The nucleus appeared large and spherical. The nucleolus in an egg cell of Type B had a considerable affinity for haematoxylin but perhaps not as much as one in an egg cell of Type A. The egg cell of Type B had a large "plump" appearance and the surrounding portion of the embryo sac had little or no affinity for stain. The ability of Type B to function in fertilization was considered doubtful but as the nucleus of Type B showed no signs of collapse, egg cells of this type may still have been functional.

In Type C the cytoplasm stained darkly or lightly but the nucleolus stained darkly. The nucleus of type C had an irregular outline but could be clearly recognized; the cell wall was usually wavy in outline. The egg cells of Type C had the appearance of partially collapsed egg cells of Type A or B. Type C was considered to be probably degenerate.

In Type D the cytoplasm stained about as in Type A, but only the nucleolus could be differentiated even in the most carefully prepared material and usually no nuclear wall could be seen. The nucleus had apparently collapsed. If a nuclear wall could be recognized at all it was shrunken and irregular in outline, the nucleolus occupying almost the entire volume of the small, shrivelled nucleus. Type D egg cells were considered to be clearly degenerate.

In describing egg cells according to Type A, B, C, and D no arbitrary line could be drawn with absolute certainty. There was a slight possibility that the differences noted between egg cells described as Types A, B, and C were due to minor variations in fixation. It seems more likely, however, that the differences in appearance resulted from genuine variability in the egg cells examined, because frequently a sample, fixed, stained, and otherwise treated by the same procedure, contained egg cells of each type. Egg cells of Types B and C were not numerous in early collections and on the basis of their appearance it was considered that these types were beginning to degenerate although this could not be verified. Type D was similar to those egg cells described as degenerating in 1957 and these very closely resembled the description given by Bradbury for degenerating egg cells in the sour cherry.

At anthesis many 8-nucleate embryo sacs were observed; however only a few of these had differentiated an egg cell (Table 4), and all of these egg cells were apparently functional. No fully differentiated embryo sacs were found with signs of degeneration at anthesis, although 20 per cent appeared to be so irregular as to be non-functional (Table 4). One such irregular embryo sac had several large nuclei located in the centre, four small ones in the chalazal end, and a very darkly staining mass of material in the micropylar end of the embryo sac. In another primary ovule more than one megaspore had divided and at least two embryo sacs were formed. Various other types of irregular embryo sacs were noted, but the proportion remained fairly constant throughout the sampling period.

Frequently the polar nuclei had not fused 1 day after anthesis and failure of the polar nuclei to fuse was also noted 2 days after anthesis. A section through an embryo sac in which the polar nuclei had not fused

PLATE IV

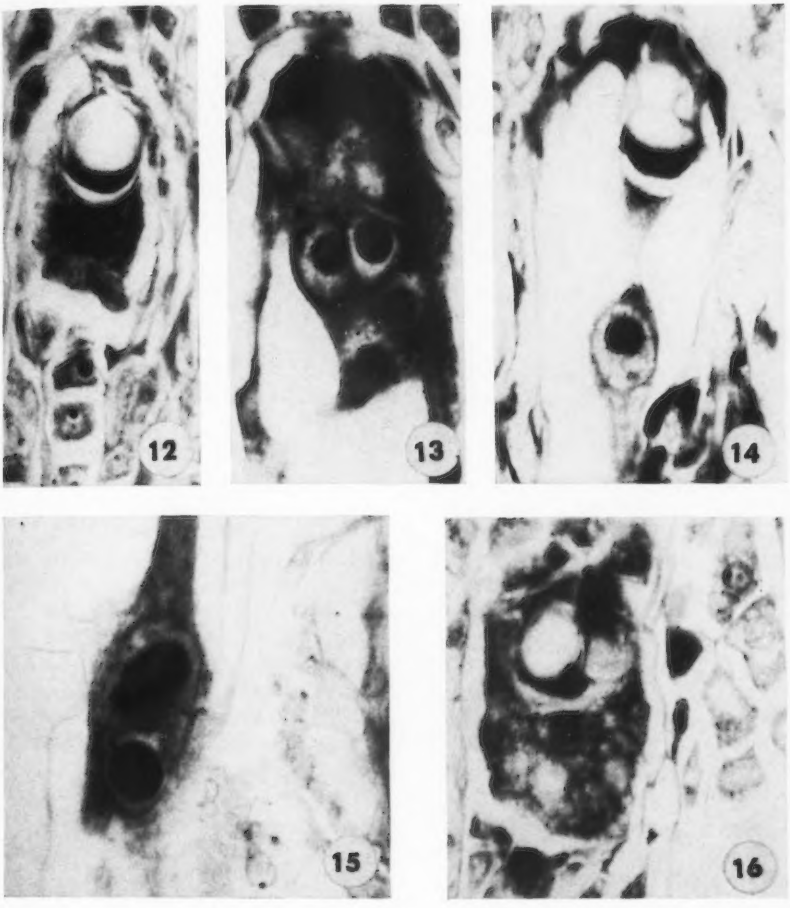

FIGURE 12. Darkly staining embryo sac with egg cell classified as Type D; 1 day after anthesis, 1958. (x 875).

FIGURE 13. Embryo sac from an unpollinated Windsor flower in which the fusion nucleus had divided several times; the adjacent section had another large nucleus like the ones illustrated here; 4 days after anthesis, 1958. (x 875).

FIGURE 14. Embryo sac containing an egg cell of Type D and an apparently functional fusion nucleus; 5 days after anthesis, 1958. (x 875).

FIGURE 15. Three nuclei of four-nucleate endosperm in embryo sac without embryo; open-pollinated Windsor collected 9 days after anthesis, 1958. (x 875).

FIGURE 16. Irregular Vista egg cell and fusion nucleus at anthesis, 1958. (x 875).

TABLE 5.—CONDITION WITHIN EMBRYO SACS OF WINDSOR SWEET CHERRY FLOWERS HAND-POLLINATED AND COLLECTED AT INTERVALS FOLLOWING ANTHESIS, VINELAND STATION, 1958*

Days after anthesis		Embryo sacs with no evidence of fertilization	Endosperm but no embryo present	Embryo but no endosperm present	Embryo and endosperm present	Both ovules shrivelled, condition of embryo sac not determinable	Total percentage with evidence of fertilization	Number of pistils examined
Pollinated	Collected							
1	8	73.6	5.3	5.3	10.5	5.3	21.1	19
1	9	50.0	50.0	—	—	—	50.0	8
1	10	60.0	—	—	—	40.0	0.0	5
	Total	65.6	15.6	3.1	6.3	9.4	25.0	32
2	8	100.0	—	—	—	—	0.0	4
2	9	91.7	8.3	—	—	—	8.3	12
2	10	75.0	25.0	—	—	—	25.0	4
	Total	90.0	10.0	—	—	—	10.0	20

*The data are expressed as a percentage of the number of pistils examined.

1 day after anthesis is illustrated in Figure 8. It is not known whether fusion of the polar nuclei is required prior to fertilization if endosperm formation is to take place.

During the first 2 days after anthesis there was a marked increase in the proportion of embryo sacs in which an egg cell had differentiated. The proportion of embryo sacs with egg cells of Types A and B never exceeded the 30.3 per cent achieved within 2 days of anthesis (Table 4).

Within 2 days of anthesis (Table 4) an egg cell could be recognized in 46.5 per cent of the embryo sacs examined. However, approximately one-third of the egg cells observed gave evidence that degeneration had begun. Only 29.1 per cent of the embryo sacs studied (Table 4) had eggs classified as Type A. Figure 12 illustrates an embryo sac in which the degeneration of the egg cell can be recognized.

In collections made 3 and 4 days after anthesis there was a sharp decrease in the proportion of egg cells of Type A accompanied by an even more pronounced increase in the proportion of egg cells of Type D. The proportion of egg cells of Type B and Type C increased rapidly throughout the 8 days in which collections were made.

In one embryo sac collected 4 days after anthesis, the fusion nucleus had undergone several divisions. A section through this embryo sac is illustrated in Figure 13.

In collections made later than 4 days after anthesis there was a further reduction in the proportion of egg cells of Type A, and an increase in the proportion of the other types of egg cell. Figure 14 illustrates a section through an embryo sac containing a degenerate egg cell collected 5 days after anthesis.

Six days after anthesis 3.1 per cent of the embryo sacs examined had not reached the 8-nucleate stage. All embryo sacs reached this stage within 8 days after anthesis. More embryo sacs had egg cells classified as B, C, or D than in any earlier collections. Nevertheless, even 8 days

after anthesis there were a few egg cells which appeared to be functional. Very few embryo sacs were observed which were so broken down that their constituents could not be recognized. In the later collections listed in Table 4, many of the embryo sacs too irregular to classify were present in ovules which were clearly degenerating. Both ovules in these instances were withered and had the appearance of secondary ovules.

Hand-pollinated Windsor Flowers, 1958

The weather in 1958, Table 2, resulted in a prolonged blooming period and only a few blossoms reached anthesis at any one time. Therefore it was impossible to hand-pollinate large numbers of flowers which reached anthesis simultaneously. The data in Table 5, although based on small numbers, indicate that pollination 1 day after anthesis was much more effective (25 per cent) in bringing about fertilization than was pollination 2 days after anthesis (10 per cent). It is not known what period of time elapsed before the sperms reached the embryo sac, but the data presented in Tables 2 and 4 explain the superiority of pollination 1 day after anthesis. A period of low temperatures (probably unfavourable to pollen tube growth) coincided with a rapid decrease in the proportion of functional embryo sacs.

These facts doubtless account for the low proportion of embryo sacs in which an embryo and endosperm were both present. A section through an embryo sac with a 4-nucleate endosperm is illustrated in Figure 15.

Hand-pollinated Vista Flowers, 1958

In collections made at the time the first pollinations of Vista were done, all embryo sacs observed had reached the 8-nucleate stage. An egg cell was differentiated in 9 and the polar nuclei had fused in 15 of the 23 embryo sacs examined at this time. Some egg cells or polar nuclei found in the Vista variety were irregular in outline, as shown in Figure 16, but in the Windsor variety the fusion nuclei showed no irregularities even in collections made more than a week after anthesis. The irregular appearance of the cells of the Vista megagametophyte was probably due to fixation rather than to degeneration since a large proportion of the Vista flowers hand-pollinated by G. H. W. Dickson in the breeding program set fruit. The poor fixation of the cells of the megagametophyte is not easily explained for mitotic figures in the minute cells of the endocarp were clearly defined.

Cytological examination of hand-pollinated flowers, collected 3, 4, and 5 days after pollination, revealed not one embryo sac that had been fertilized. No differences could be distinguished between hand-pollinated and unpollinated flowers collected on the same date. Tukey (10) observed very small embryos in sweet cherry 4 or 5 days after full bloom. Bradbury (1) observed various steps in the process of double fertilization in sections of pistils collected 3 or 4 days after full blossom. While neither Bradbury nor Tukey mention the temperatures prevailing during the period in which they took their samples, it is quite possible that their temperatures were not as low as those recorded in Table 2. Probably the Vista flowers were collected too soon after hand-pollination for fertilization to have taken place at the prevailing temperature.

DISCUSSION

It has been shown that, at anthesis, in both 1957 at Columbus, Ohio, and 1958 at Vineland Station, Ontario, a sufficient number of apparently functional embryo sacs were present for a commercial crop of fruit, providing a large enough proportion of functional embryo sacs became fertilized. Delayed development of the megagametophyte and irregularities in its development were not numerous enough to alone explain reduced fruit set in Windsor sweet cherry.

Average maximum and average minimum temperatures in 1957 were approximately 20° F. higher than in 1958. Apparently the lower temperatures in 1958 inhibited the development of the megagametophyte more than the development of the external flower parts, since on the whole megagametophytes were more advanced at anthesis in 1957 than at anthesis in 1958.

Results of hand-pollinations of both the Windsor and Vista varieties indicated that collections were made before fertilization had an opportunity to occur in many of the embryo sacs. Although there are reports (1,10) that fertilization has been observed within 3 or 4 days of anthesis, it is apparent that such reports cannot be generally applied without consideration of the prevailing temperatures. After pollination it would be well to wait at least 7 days before collecting the ovules. A somewhat longer interval may be advisable when the weather is cool.

Degeneration of egg cells increased following anthesis and it is apparently in this phenomenon that the explanation of unsatisfactory fruit-set largely lies. Beginning at anthesis in 1957, and within 2 days of anthesis in 1958, the proportion of functional embryo sacs rapidly decreased. Therefore, it is probable that, the later pollination is delayed, the lower will be the proportion of embryo sacs in which fertilization takes place. In an experiment in which flowers were pollinated at intervals following anthesis (Table 5), pollination 1 day after anthesis was much more effective in accomplishing fertilization than was pollination 2 days after anthesis. The length of time required for the pollen tube to grow down the style and for the sperms to reach the embryo sac is not known. However, the length of this period could place additional emphasis on the importance of the earliest possible cross-pollination so the maximum number of embryo sacs may be fertilized. It may be that on some occasions in the past favourable conditions for pollination occurred only during a period when the external appearance of the flower was still normal but when a large proportion of the embryo sacs were non-functional. It is also possible that in certain instances the proportion of functional embryo sacs was much lower than was observed in this study. The importance of cross-pollination immediately at anthesis in the light of present knowledge, should guide grower practices in order to ensure a commercial set of fruit in the sweet cherry.

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THE EFFECT OF SWATHING AT DIFFERENT STAGES OF MATURITY ON THE BUSHEL WEIGHT AND YIELD OF FALL RYE[1]

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ABSTRACT

Tests were conducted over a 4-year period to determine the earliest stage of maturity at which fall rye could be swathed without loss of bushel weight or yield.

Cutting commenced when the kernel moisture content of the standing crop approximated 50 per cent and continued until the kernel moisture reached 14 per cent or until a maximum of 20 plots (15 in 1955) had been cut. The weight per measured bushel, 1000-kernel weight, and yield in bushels per acre were determined at the time of picking up the swath with the combine.

An analysis of variance of the data indicated that fall rye may be swathed at a stage of maturity defined by a kernel moisture content of 45 per cent without affecting yield or bushel weight. Correlations between kernel moisture at the time of swathing and bushel weight, 1000-kernel weight and yield indicated that generally a significant negative association existed to the 40 per cent moisture level but the relationship was not significant at the later stages of maturity.

INTRODUCTION

The recommended procedure, and the usual practice for harvesting the fall rye crop in Western Canada, is swathing rather than straight combining. This grain usually matures 10 days to 2 weeks in advance of other crops and can be completely harvested before barley or wheat have reached the harvest stage of maturity.

Uniformity of maturity has always been accepted as one of the prerequisites for straight combining. This stage is particularly difficult to recognize in a field of standing rye because of the tillering habit of the plant and the extended flowering period. Shattering is a characteristic of the commercial varieties of rye grown in Western Canada and late swathing or straight combining aggravates this condition. The shattered seed volunteers freely with the result that subsequent crops are contaminated.

A study with Antelope rye was conducted at the Experimental Farm, Swift Current, Saskatchewan, during the years 1955 to 1958, inclusive, to determine the earliest stage of maturity at which fall rye could be swathed to produce maximum yields without loss of quality. The results of these studies are reported here.

REVIEW OF LITERATURE

Gregory and Purvis (3) and Hatcher and Purvis (4) have studied the growth habits of rye kernels removed from the ear at various stages of maturity and report that even very immature seeds produce healthy

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TABLE 1.—KERNEL MOISTURE CONTENT WEIGHT PER MEASURED BUSHEL, 1000-KERNEL WEIGHT AND YIELD DATA, 1955-1958

Swathing tests with Antelope Rye
Swift Current, Saskatchewan

1955					1956					1957					1958				
Swathing date	K.M.C. at swathing	W.M.B.	1000-kernel weight	Yield per acre	Swathing date	K.M.C. at swathing	W.M.B.	1000-kernel weight	Yield per acre	Swathing date	K.M.C. at swathing	W.M.B.	1000-kernel weight	Yield per acre	Swathing date	K.M.C. at swathing	W.M.B.	1000-kernel weight	Yield per acre
	%	lb.	gm.	bu.		%	lb.	gm.	bu.		%	lb.	gm.	bu.		%	lb.	gm.	bu.
July 21	60.5	49.1	13.45	22.4	July 31	46.5	58.5	22.80	19.5	July 19	47.0	59.2	21.96	26.7	July 17	46.2	58.1	17.40	15.8
July 22	58.5	50.0	13.98	22.4	Aug. 1	44.5	58.5	22.35	19.0	July 22	42.1	59.4	23.09	29.9	July 18	44.7	57.8	17.83	11.3
July 25	56.0	54.0	16.35	27.1	Aug. 2	40.6	56.4	22.95	18.3	July 23	41.9	59.4	22.98	30.5	July 21	35.5	58.6	18.02	12.7
July 26	53.6	54.8	17.95	27.7	Aug. 3	37.4	58.9	23.02	24.0	July 24	36.0	59.1	22.74	29.6	July 22	21.8	58.3	18.55	11.2
July 27	53.3	54.6	20.30	23.1	Aug. 6	33.1	55.5	23.20	24.1	July 25	29.2	59.6	22.36	32.0	July 24	21.6	58.8	18.94	13.6
July 28	49.6	55.3	20.85	31.1	Aug. 7	25.6	55.0	22.95	23.6	July 26	24.2	58.8	22.70	33.2	July 25	19.3	57.8	17.32	9.9
July 29	51.6	55.8	20.48	34.5	Aug. 8	22.3	56.9	23.12	22.3	July 27	18.9	58.9	23.26	30.5	July 28	17.8	58.3	17.70	10.7
Aug. 1	47.0	56.6	22.52	31.6	Aug. 9	23.9	56.0	23.57	22.7	July 29	15.7	58.5	22.82	33.3	July 29	14.7	58.3	17.95	12.1
Aug. 2	45.4	57.1	22.65	32.0	Aug. 10	22.5	55.9	22.92	24.0	July 30	14.4	58.2	23.46	30.8	July 30	13.2	58.5	18.50	15.6
Aug. 3	44.7	57.1	24.40	34.3	Aug. 13	19.0	54.1	22.56	22.6										
Aug. 4	42.8	57.6	24.53	34.8	Aug. 14	22.0	51.1	22.51	27.5										
Aug. 5	40.9	57.3	23.22	35.1	Aug. 15	19.3	50.7	22.19	25.0										
Aug. 8	28.6	57.1	25.90	36.7	Aug. 16	19.2	52.5	22.91	31.0										
Aug. 9	30.2	57.1	25.30	35.7	Aug. 17	18.6	53.4	22.57	27.9										
Aug. 10	23.6	56.8	26.70	33.3	Aug. 20	20.0	52.4	22.44	23.4										
					Aug. 21	19.6	54.9	22.29	25.7										
					Aug. 22	18.5	55.0	21.12	25.6										
					Aug. 23	19.5	51.8	22.24	25.1										
					Aug. 24	17.3	53.1	21.99	28.2										
					Aug. 27	21.0	51.1	21.56	29.2										
L.S.D. (P=0.05)	2.88	1.16		4.89		5.14	2.88	0.91	5.06		3.35	N.S.	N.S.	N.S.		3.57	N.S.	N.S.	N.S.
S.E.M.%	2.21	0.73		5.58		7.13	1.87	1.43	7.32		3.85	0.86	2.51	4.78		4.71	0.60	2.17	16.06

plants. Nutman (5, 6) states that the final stage of embryo-sac development is complete about the twentieth day from anthesis and must have reached this degree of growth before grain detached from the ear is able to complete its development. Nuttonson (7) describes the progression of the ripening of the rye grain as the milky-ripe stage, containing 40 to 60 per cent moisture, the dough stage 30 to 40 per cent, the wax-ripe 20 to 25 per cent, and full ripe 15 to 20 per cent depending on humidity and temperature in the area. He further states that a timely harvest is imperative if loss of grain through shattering is to be avoided.

PROCEDURE

Antelope rye, representing a typical commercial variety, was used in all the tests. The procedure followed was that outlined by Dodds (1) with wheat, and Dodds and Dew (2) with barley. Stage of maturity was defined by a determination of kernel moisture content for each day of sampling, the previous work with other grains having specified that the range of moisture should be between 50 and 14 per cent. The studies of Nutman (5, 6) indicated to the writer that the early limits of ripening at Swift Current would occur a few days after the stage described by him.

The design used for this field plot experiment consisted of four randomized blocks of 20 plots each in all tests, except 1955 when only 15 plots were available. Swathing started when the grain approximated 50 per cent kernel moisture and continued, weather and field conditions permitting, until the grain reached 14 per cent moisture or less. Cutting was done with a self-propelled swather, the acreage for calculation purposes being one swath wide by the length of the plot or approximately 1/35 acre. A second adjacent swath was cut for the sampling only, thus not disturbing the first swath which was used for yield.

Kernel moisture determinations were made immediately after cutting from samples of threshed, cleaned grain. Samples collected daily from each swath were threshed and dried to determine the rate of drying in the swath.

The swaths were threshed with a combine when the kernel moisture content reached 14 per cent or less. Yield in bushels per acre was calculated from the recorded weight of grain from each plot on the basis of the standard 56-pound bushel. The weight per bushel and 1000-kernel weights were recorded without further processing of the samples.

RESULTS AND DISCUSSION

The bushel weight, 1000-kernel weight, and yield data for the test period 1955 to 1958, inclusive, are shown in Table 1.

Swathing was started at a very early stage of maturity in 1955. Bushel weight increased until the kernel moisture content reached 47 per cent after which stage there was no significant difference in this characteristic. The weight per 1000 kernels was determined from a bulk sample of the four replicates and could not be analysed statistically. Yield improved until the kernel moisture content dropped to 51.6 per cent with no significant increase in later stages of maturity.

TABLE 2.—TABLE OF CORRELATION COEFFICIENTS

Correlation between kernel moisture and weight per bushel, 1000-kernel weight and yield, Antelope Rye

Swift Current, Saskatchewan

Year	Kernel moisture correlated with weight per bushel			Kernel moisture correlated with 1000-kernel weight			Kernel moisture correlated with yield		
	Range of kernel moisture	Number of tests	r	Range of kernel moisture	Number of tests	r	Range of kernel moisture	Number of tests	r
	%			%			%		
1955	60.5 – 23.6	15	−.713**	60.5 – 23.6	15	−.895**	60.5 – 23.6	15	−.759**
	53.3 – 23.6	11	−.394	49.6 – 23.6	10	−.609	53.3 – 23.6	11	−.565
1956	46.5 – 21.0	20	+.753**	46.5 – 21.0	20	+.350	46.5 – 21.0	20	−.727**
	33.1 – 21.0	16	+.422				37.4 – 21.0	17	−.356

**Significant at P = .01

The 1956 crop was fairly severely winterkilled and the consequences are reflected in both bushel weight and yield. The initial growth was light but summer rains encouraged second growth which headed early in August. Weight per bushel decreased with the advance of time due to the presence of light immature kernels and became significantly poorer after the 22.3 per cent kernel moisture stage. The weight per 1000 kernels indicated a trend to increase and reached a maximum on August 9. The decline following that date can be attributed to the second-growth material. The increase in yield from the beginning of the test until August 13 was not significant. After this stage of maturity enough of the grain from second growth was retained by the threshing mechanism of the combine to produce a marked increase in yield. These data illustrate very clearly how second growth can delay harvesting operations by maintaining a high kernel moisture content. Late harvesting can produce a low quality product under such conditions.

No significant differences in bushel weight, 1000-kernel weight, or yield were established between samples harvested at different dates either in 1957 or 1958. The high experimental error for yield in 1958 expresses the high variability of yields that was caused by a very severe drought during the growing season.

Correlation coefficients between kernel moisture content at the time of swathing and bushel weight, 1000-kernel weight and yield were calculated using means of the four replicates of these characteristics. The results are shown in Table 2.

The results from the 1955 and 1956 data followed a pattern similar to that found with other grains. The climatic conditions in 1955 were conducive to a normal plant growth and development, kernel moisture content being highly significantly and negatively correlated to bushel weight, 1000-kernel weight, and yield for the full range of the test. As maturity advanced this association gradually lessened and ceased to exist at about the 53 per cent kernel moisture level. The presence of light weight, immature kernels, caused by second growth, is reflected in the 1956 data when kernel moisture is highly significantly and positively correlated to bushel weight and negatively correlated to yield for the full test. This association was not apparent in the stages of maturity after the kernel had reached 37.0 per cent moisture.

The 1957 and 1958 data represent results that can be expected during seasons of near drought and the patterns formed for these years are practically identical. The variance analysis indicated no significant differences for bushel weight, 1000-kernel weight and yield within the ranges of the tests, so no further comparisons were made.

The quality of the swath formed by cutting at different stages of maturity was observed during the test. It was noted that, at even the very early stages, the swath was well bound and remained adequately supported on the stubble.

The data collected on the rate of drying in the swath indicated that rye requires a longer period in the windrow than other grains in order to reach the minimum moisture level for safe storage. Grain cut at a moisture

content of 40 to 45 per cent required 7 to 9 days' drying time in the swath. This stage of maturity occurred 9 to 10 days in advance of the stage usually recognized as suitable for straight combining.

CONCLUSIONS

1. The swathing of fall rye can be started at an early stage of maturity defined by a kernel moisture content of 45 per cent without significant loss of bushel weight or yield.
2. This stage of maturity occurs 9 to 10 days in advance of the condition at which straight combining can be started in a year when dry conditions exist during the growing season and earlier under more favourable conditions.
3. Rye requires a longer period in the swath than other grains in that 7 to 9 days of drying are necessary to reduce the moisture to a condition suitable for threshing with the combine.
4. The grain matures well in the swath and is well supported on the stubble, thus reducing losses from wind, rain, or hail.

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NEAR-WILT OF PEAS IN ONTARIO

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ABSTRACT

It has been found that the near-wilt disease of peas, caused by *Fusarium oxysporum f. pisi* race 2 (Linford) Snyder & Hansen, is now present in the canning districts of Ontario. Conclusions as to the identity of the fungus associated with the wilting condition in field plants have been based upon a study of the differential disease-response of the pea varieties Little Marvel, W.R. Perfection and New Era, the histology of infection in the susceptible pea host, and the characteristics of the fungus when in culture. The capacity of the fungus to establish a symptomless host-parasite relationship with certain legumes not normally regarded as susceptible to the near-wilt pathogen has been demonstrated. The possible role of such "non-susceptible hosts" in perpetuating the fungus between pea crops has been discussed.

INTRODUCTION

The name "near-wilt" was introduced by Snyder and Walker (8) to describe a wilting condition in peas caused by race 2 of the vascular pathogen *Fusarium oxysporum f. pisi* (Linford) Snyder and Hansen. Historically, the name has served to separate this syndrome from another known as "wilt", the casual agent of which is a related, but distinct strain of the fungus, namely race 1.

The occurrence of near-wilt of peas in Ontario was first reported by MacNeill (4) in 1956. Since this disease has proved to be very serious in certain of the canning areas of the United States, the likelihood of its becoming a threat to the pea industry of Ontario was immediately recognized. The present investigation was initiated primarily to confirm the earlier field diagnosis of the wilting condition in peas, but other factors which appear to be important in the epiphytology of the disease in the local canning crop districts have also been considered.

SYMPTOMS

Near-wilt, as observed in Ontario, begins as a yellowing of the leaves and stem of the pea plant just before it begins to blossom. Later, the leaflets wilt, become leathery in texture and roll inward. Plants may persist in a semi-wilted condition until harvest. Such near-wilt plants often mature earlier than healthy plants in the same field and form hard, starchy seeds which contribute to a very uneven grade of shelled peas.

Accompanying these outward symptoms, there may be a marked orange to orange-brown discoloration of the vascular tissues of the pea stem extending for some distance above the soil line. Usually the cortical tissue shows no evidence of invasion, but occasionally it becomes noticeably decayed and the mycelium of the pathogen may then be visible on the surface of the stem at the soil line.

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ETIOLOGY

Repeated isolations were made from samples of pea plants showing symptoms of near-wilt. These samples were collected from commercial fields of canning peas late in the season, generally just prior to harvest. Segments cut from the bottom portion of each internode of the stem of such plants were surface sterilized in 0.1 per cent mercuric chloride solution for 5 minutes, then rinsed in sterile, distilled water and plated on acidified, potato sucrose agar (P.S.A.). After a period of incubation at 25° C. the plates were examined, and fungi which appeared to have their origin in the vascular elements of the stem were selected for study (Figure 1). A species of *Fusarium* was found to be consistently associated with such samples. Later it was found convenient to obtain this *Fusarium* from field material simply by washing the infected plant stems in briskly running water and incubating them between folds of moist paper towels; within 48 hours the fungus appeared in a luxuriant growth on the surface of the stems (Figure 2). This same species of *Fusarium* could also readily be obtained by growing the susceptible pea variety W.R. Perfection in soil from fields with a record of the disease, then incubating, as above, the stems of plants which developed the characteristic near-wilt symptoms. This technique, when carried out at soil temperatures ranging from 25°–28° C., proved fairly selective for the pathogen under consideration.

The consistent association of this *Fusarium* with the wilting condition of peas, the systemic nature of the fungus in its host, the rather obvious specialization of the pathogen to peas, and its cultural characteristics as described below, suggested that the organism under study was *Fusarium oxysporum f. pisi* (Linford) Snyder and Hansen.

CULTURAL CHARACTERISTICS

Stock cultures of the isolates of *F. oxysporum f. pisi* obtained from peas were maintained in soil-tubes and on soil-extract agar. Under such conditions the fungus appeared to remain stable and, when subsequently plated upon P.S.A., gave no indications of having sectored, mutated or changed its habit in any way. Nor was there any variation in pure culture to suggest the cultivation of a mixture, and in no instance did the fungus lose its pathogenicity when tested on its susceptible pea host.

Growth in culture, on P.S.A., was light and raised, characteristic of a wild type (5). Pigmentation of the medium, while influenced by the intensity of the light, carbohydrate source, and pH of the medium, was generally lavender which deepened to purple with age. On P.S.A. the fungus grew best at 28° C., the growth curve rising gradually to this point from about 18° C. and falling off quickly between 28 and 30° C.

PATHOGENICITY TESTS

Pathogenicity tests were conducted in a controlled-climate chamber equipped with a panel of incandescent and fluorescent lights to give a total illumination of approximately 800 foot-candles at bench level (7). Air temperature and photo-period were regulated according to the require-

FIGURE 1. Stem segments from the third, sixth, ninth and tenth internodes of W.R. Perfection peas yield *F. oxysporum f. pisi* after 3 days' incubation at 25° C.

FIGURE 2. Diseased pea stems after incubation between moist paper towels readily yield the pathogen.

ments of the experiment. Under these conditions no difficulty was experienced in growing satisfactory test plants. In certain instances, the soil temperature was manipulated independently by using a battery of Wisconsin temperature tanks installed within the controlled-climate chamber.

Experimental plants were grown in sand-culture in waxed, 8-ounce paper cups with drainage holes punched in the bottom. Inoculation was carried out by incorporating corn-meal-sand inoculum at the rate of 2 per cent by volume into the sand medium in which the plants were growing, or by root-dipping the plants in a heavy spore suspension of the pathogen and then re-potting them in the waxed containers.

Five isolates of *F. oxysporum*, from field plants showing the characteristic symptoms of near-wilt, were tested separately for their pathogenicity to peas. These isolates were obtained by "mass transfer" of mycelium and spores from incubated stem segments rather than by the culture of single spores; this technique avoided any undue tendency to select heavily sporulating strains, or other atypical forms. Occasionally the stocks were replenished by inoculating susceptible peas with the isolates and then recovering them after they had survived host passage. All five isolates proved to be pathogenic on the pea variety W.R. Perfection, although there was some indication that they were not equally aggressive. Subsequent studies were carried out using Isolate 4, which was not only highly aggressive, but was obtained from a stem segment some 8 inches above the soil line, a region in which there could be little doubt as to the systemic nature of the fungus.

DIFFERENTIAL HOSTS

The evidence from field observations and laboratory tests suggested very strongly that the disease under study was near-wilt. For instance, symptom expression in the field was strikingly similar to that reported by Snyder and Walker (8) for this disease; moreover, the pathogen inhabited a vascular locus in its host; and the pea variety W.R. Perfection was highly susceptible. Indeed, since the latter variety had been released to the canning trade as resistant to *F. oxysporum f. pisi* race 1, there could be little doubt that its susceptible reaction was due to a distinct race of *F. oxysporum f. pisi*, presumably race 2, the near-wilt pathogen.

To supplement this evidence, an attenpt was made to delimit more specifically the pathogenic nature of the Ontario isolate by means of differential host reactions. For this purpose, New Era, a pea variety originated by Hagedorn (2) to combat the threat of near-wilt in Wisconsin, was incorporated into a series of pathogenicity tests along with the susceptible variety W.R. Perfection.

Groups of New Era and W.R. Perfection plants grown in paper cups were inoculated when they were 12 days old by dipping the roots in a heavy spore suspension of the *Fusarium* Isolate 4 referred to above. Following this, the plants were returned to the controlled-climate chamber and incubated for 2 weeks at 23° C. At the end of this period all the W.R. Perfection plants were yellowed and severely wilted; the New Era plants, on

TABLE 1.—DIFFERENTIAL RESPONSE OF PEA VARIETIES TO ISOLATES OF *Fusarium oxysporum f. pisi*

	Susceptible to Races 1 and 2	Resistant to Race 1, susceptible to Race 2	Resistant to Races 1 and 2
	Little Marvel	W.R. Perfection	New Era
Isolate 14	Wilt	No wilt	No wilt
Isolate 12	Wilt	No wilt	No wilt
Isolate 4	Wilt	Wilt	No wilt

the other hand, retained their normal green foliage and showed no evidence of near-wilt symptoms. Within 5 weeks of inoculation nearly all of the W.R. Perfection had died, but the New Era remained disease-free.

To check further on the specificity of the differential response of New Era and W.R. Perfection, the action of Isolate 4 was compared with that of two isolates of *F. oxysporum f. pisi* race 1. Of these latter two, Isolate 14 was supplied by G. M. Armstrong, of Clemson Agricultural College, South Carolina, as a typical culture of the pea wilt organism, while the other, Isolate 12, was obtained from stem segments of a Little Marvel pea which was showing severe symptoms of wilt in a local home-garden. When these three Fusaria were used as inoculum on the pea varieties New Era, W.R. Perfection and Little Marvel, it was obvious, on the basis of the response of the plants, that two distinct physiologic races were present, the susceptible reaction of W.R. Perfection to Isolate 4 serving to separate the latter pathogen from the other two used in the experiment (Table 1). Accordingly, it was concluded that Isolate 4, representative of the pathogen under discussion in this paper, was, undoubtedly, *F. oxysporum f. pisi* race 2.

EFFECT OF SOIL TEMPERATURE ON DISEASE EXPRESSION

Fifteen-day-old W.R. Perfection plants were root-dipped in a spore suspension of the *Fusarium* Isolate 4, then re-potted into sterilized soil and placed in Wisconsin temperature tanks maintained at 19, 23 and 28° C. in the 23° C. controlled-climate chamber.

Marked differences were noted in the intensity of the disease which developed at the various soil temperatures. Within 20 days of inoculation, symptom expression was extremely severe at 28° C., and only slightly less severe at 23° C. At 19° C., however, an additional week of incubation was required to reach a comparable stage of disease development, and at this temperature there was a strong tendency for plants to persist and even, in many instances, to set pods.

Stem segments from plants which had become infected at the various soil temperatures readily yielded the *Fusarium* when plated on P.S.A. It was found, however, that the intensity of the symptoms did not always reflect the invasive activity of the pathogen; for example, plants not yet

showing severe effects of attack at 19° C. were often as extensively colonized in the stele as those at 28° C. which were more noticeably affected. These observations suggest that the effect of soil temperature is primarily one which acts through the host; the pea, a low-temperature plant, apparently is unable to restrict the wilting factor when it is in an environment unfavourable for vegetative growth.

In naturally infested soil, the relative effects of temperature upon the development of disease followed a pattern similar to that reported above, but the onset of disease and its subsequent development were much less rapid than observed previously for the artificially infested soil. At 28° C., the first symptoms of near-wilt did not appear until the plants were beginning to blossom, some 12 days later than for plants in artificially infested soil, and a comparable delay was observed at 23° C. and 19° C. Apparently, the inoculum potential of the naturally infested soil was relatively low, but no attempt was made here to analyse the factors which might have an influence on this potential.

HISTOLOGY

F. oxysporum f. pisi race 2 is acknowledged to be a vascular parasite of its pea host (8), and the isolation of this fungus from segments of diseased stems taken 8–12 inches above the soil line supports this view. But, since efficacy of a surface disinfectant might be questioned, the systemic nature of the pathogen was confirmed by examination of longitudinal sections from stems of diseased W.R. Perfection plants. These sections were made free-hand with a razor and mounted in lactophenol-cottonblue. In such preparations, the mycelium was clearly visible, extending from cell to cell in the vascular tissue, and in many instances was noted to produce spores within the vessels. In stems with a sound cortex, the fungus was confined to the vascular bundles, but in stems in which the cortical tissue had undergone some disintegration the mycelium had invaded the cortex as well as the stele.

An histological examination was also made of the variety New Era which, as reported earlier, had proved to be resistant to near-wilt. In preparations from plants which had been exposed to the pathogen at both 23 and 28° C., the fungus could be detected in the xylem tissue as far as 5 inches above the seed, even though the plants were showing no external indications of attack. This type of invasion was not as extensive as that found in W.R. Perfection, and the tortuous, knotted nature of the mycelium in the resistant host was in marked contrast to the unrestricted type of development noted in the susceptible variety. These observations indicate that the possibility of New Era harboring the pathogen and so maintaining the inoculum potential in areas where this variety is grown should not be overlooked in any consideration of the epiphytology of near-wilt.

HOST RANGE STUDIES

The possibility that certain crops, especially legumes, used in rotation with peas would harbor the near-wilt organism and thereby maintain a substantial level of the fungus in the soil, was explored in a series of infection

experiments using alfalfa, red clover, snap bean and soybean. All four of these crops are grown in Ontario, and often find a place in the rotation program of producers of canning peas.

Experimental plants were grown in sand-culture, as before, and maintained at 23° C. in a controlled-climate chamber both prior to and after inoculation. The varieties used were Vernal (alfalfa), Redon (red clover), Scotia (snap bean) and Mandarin (soybean).

Under these conditions the relatively fast growing snap beans and soybeans were ready for inoculation when $2\frac{1}{2}$ weeks old, at which time they were root-dipped in a spore suspension of Isolate 4 of *F. oxysporum f. pisi* race 2, then re-potted and incubated for another 2 weeks. Uninoculated plants were root-dipped in tap water and maintained under similar environmental conditions to serve as checks. At the end of the 2-week incubation period, both the snap beans and soybeans had made extensive vegetative growth without any indication of disease. However, when stem segments were surface sterilized with mercuric chloride solution and plated on P.S.A., the fungus could be recovered from the lower stem segments of both kinds of plants. In the snap bean, particularly, invasion was extensive and the fungus was obtained from stem tissue approximately 3 inches above the attachment of the cotyledons.

The slower growing red clover and alfalfa plants were inoculated when they were 4 weeks old and platings of stem segments were made at the end of a 5-week period of incubation at 23° C. Again, as reported for the snap beans and soybeans, there were no symptoms suggesting that the plants had been invaded. Yet, the fungus was recovered from the alfalfa stems at a height of 6 inches above the soil line and from nearly all of the shoots of the crown, and in clover the isolations were positive from stem segments as high as 3 inches above the soil line.

These studies, while carried out under substrate conditions in which the organism was free from natural microbiological associative effects, suggest that *F. oxysporum f. pisi* race 2 may invade plants other than those generally considered as susceptible. This might be of considerable importance in the survival of the organism between pea crops.

DISCUSSION

The existence of near-wilt of peas in the Ontario canning crops districts has now been confirmed. As yet the disease may be said to be sporadic and often confined to small areas in a field. However, in several instances the disease has assumed major proportions, and fields involving several acres have been affected (4). At the moment, the distribution of near-wilt appears to be in a discontinuous pattern throughout the province; this, undoubtedly, is due to the seed-borne nature of the pathogen which would permit many separate introductions. Field observations suggest, on the other hand, that once an area has become infested, the inoculum potential remains high, and the disease-area gradually increases in size. If it should be found that the local environment favours the survival and persistence of the pathogen, then the problem of introducing a pea variety

with resistance to near-wilt becomes an urgent one. At the present time almost all of the commercially acceptable pea varieties grown in Ontario are of Perfection parentage, and as a consequence are highly susceptible to this disease.

The effect of soil temperature on the Ontario isolate of *F. oxysporum f. pisi* race 2 is similar to that reported by Virgin and Walker (9) for the Wisconsin isolate of the near-wilt pathogen. The intensity of the reaction of the pea plant to infection at 28° C. appears, on the basis of the present histological evidence, to be due to the effect of this high temperature upon the host plant which is growing somewhat beyond its optimum of 16-20° C., rather than to an accelerated aggressiveness of the fungus operating at its vegetative optimum of 28° C. At 19° C., damage by the pathogen to the pea plant is relatively light, although invasion of the host may be fully as extensive as at the higher temperatures of 23 and 28° C. where disease is severe. Indeed, at 19° C. infected plants often persist until mature and in this regard may be quite comparable to diseased plants found under field conditions. A correlation, then, appears to exist between these laboratory findings and the recorded field data (8, 9). In the field, early maturing varieties suffer little from near-wilt, having the advantage, presumably, of an entire season of favourable soil-temperature conditions. In contrast, near-wilt may be prevalent in the late-maturing varieties which are subjected to the higher, above-optimum soil-temperatures.

In still another regard, the effect of temperature must be considered in the epiphytology of near-wilt. The resistant New Era has been shown to harbor the fungus without showing any outward symptoms, but it is probable that, should high soil-temperatures unfavourable to this variety occur in the field, the plant would be disposed toward a susceptible reaction. So far as can be determined there is no record of the field resistance of New Era breaking down under the circumstances suggested here, but it would seem wise to test such resistant plants under a broad range of temperature conditions before adopting them for general use on a commercial scale.

The isolation of the near-wilt fungus from a systemic locus in stems of the legumes alfalfa, red clover, snap bean, and soybean, is evidence that the organism can invade plants other than those considered as usual hosts (formae suscept). This phenomenon has been observed by other workers for several of the wilt *Fusaria*. Armstrong and Armstrong (1) found that *F. oxysporum f. batatas*, the sweet potato pathogen, invades the stem of such plants as cotton, soybean and tomato growing in sand-culture, and that, in the field, the cotton plant may be extensively invaded by this forma without developing any external symptoms. These workers use the term "non-susceptible host" to describe such a plant. More recently, Hendrix and Nielson (3) have demonstrated that the invasion and infection of crops other than the forma suscept may occur in greenhouse sand-culture with several formae of *F. oxysporum*, and also that the organism could be recovered from a range of symptomless hosts when the latter were growing in naturally infested field soil. In the present study, no attempt has been made to investigate the range of "non-susceptible hosts" beyond the legume crops, nor has it been demonstrated here that *F. oxysporum f. pisi* race 2 will invade either alfalfa, red clover, snap bean or

soybean under field conditions. Nevertheless, a strong probability exists that certain crops acting as symptomless hosts might tolerate invasion and so permit the pea pathogen to perpetuate itself. The value, then, of legumes, and possibly other crops too, in a rotation program with peas is open to question until such time as the natural relationship of these crops with the near-wilt fungus has been established.

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SURVEYS OF AIR-BORNE RAGWEED POLLEN IN CANADA WITH PARTICULAR REFERENCE TO SITES IN ONTARIO[1]

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ABSTRACT

Results from 164 pollen collecting stations across Canada indicate that the heaviest concentration of ragweed pollen in the air is in southern Ontario and Quebec. The northern limits of this heavy ragweed pollen concentration extend from the base of the Bruce Peninsula to the St. Lawrence River about midway between the mouth of the Saguenay River and Quebec City. Zones of different intensities of air-borne ragweed pollen in Ontario are determined from investigations initiated in 1950.

INTRODUCTION

One of the most important biological impurities of the air in eastern North America is the pollen of ragweeds, *Ambrosia* spp. It is now claimed that 2.5 to 3 million hay fever sufferers in eastern North America are sensitive to the pollen of these plants.

The first study of air-borne pollen in Canada was carried out in 1930 by Detweiler and Hurst (9) at Toronto. In their 3-year study large quantities of ragweed pollen were recorded during late August and September. From 1930 to 1932 Durham (10) established pollen collecting stations at Prince Albert, Winnipeg, Port Arthur, Toronto, Ottawa and Montreal. He collected only 6 ragweed pollen grains on slides exposed at Prince Albert, Saskatchewan. At Winnipeg and Port Arthur the average amount of pollen on the slides was much less than at Toronto, Ottawa and Montreal. Durham concluded that more ragweed pollen was present in the air immediately north of Lake Erie than in all the rest of Canada. La Rush (14) at Toronto, MacDermot and Howell (15) at Montreal, and Hughes (12) at Hamilton published early reports on the pollen problems of their respective localities, and in 1945 Campagna (4) published a comprehensive paper on ragweeds and their pollen in the Gaspé Peninsula. Subsequent to this early work, investigations have been intensified and expanded to include all parts of Canada where ragweeds might occur. Air-borne pollen surveys by Kennedy (13), Walton and Dudley (17), and by the author (3) have revealed that little ragweed pollen occurs in western Canada with the exception of southern Manitoba. In 1955, Collins-Williams and Best (8) published a more recent pollen survey report for Toronto, Ontario. Campagna in several papers (5, 6, 7) has reported on air-borne ragweed pollen surveys for Quebec and the Atlantic Provinces and recently this work has been expanded in New Brunswick by C. W. Argue, of Fredericton. A booklet "*Canadian Havens from Hay Fever*" (3), revised for the fourth time since 1952, contains the ragweed pollen air-indexes for localities across Canada where the incidence of pollen has been investigated.

[1]Contribution No. 2 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

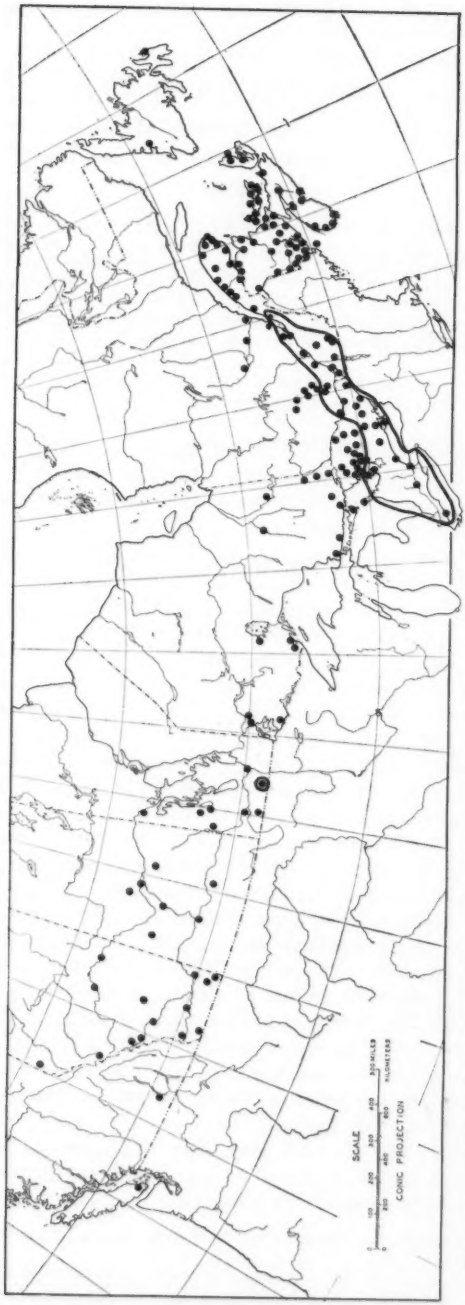

FIGURE 1. Sites in Canada where ragweed pollen air-indexes have been recorded. The areas inside the heavy lines include stations with ragweed pollen air-indexes above 10.

Pollen data from only 6 stations were available to Durham in 1933 when he discussed the general distribution of air-borne ragweed pollen in Canada. At the present time data are available from 164 stations and the general picture of ragweed pollen in Canada can now be described more precisely. None of the papers mentioned above has dealt with the ragweed pollen situation in Ontario on a broad scale and this paper introduces the results of investigations in that Province from 1950 to 1958. An attempt has been made to indicate zones of different intensities of ragweed pollen in that area.

METHODS

The devices and techniques used for collecting air-borne pollen in Ontario were those approved by the Committee on National Pollen Survey of the American Academy of Allergy (1). These same devices and techniques had been used for most stations in other parts of Canada. The procedure consists essentially of a 24-hour exposure of glycerine jelly-coated slides in a standard air-sampling device, counting of ragweed pollen over a unit area of 1 square centimetre, and converting centimetre counts into approximate volumetric equivalents by using standard factors. The devices were placed well above ground to permit complete air movements on all sides. The intensity and duration of the ragweed pollen season at any particular site can be expressed by a single index figure (11). This index figure is derived from the formula $\frac{A}{200} + \frac{B}{100} + \frac{C}{-}$ where A is the total number of ragweed pollen grains per cubic yard of air for the ragweed season, B is the total pollen per cubic yard of air for the peak day and C is the number of days with 25 or more pollen grains per cubic yard of air. Sites with indexes above 10 are not recommended for hay fever sufferers, those between 5 and 10 are fairly good, below 5 are good and below 1 are excellent.

RESULTS

Figure 1 shows all the sites in Canada where ragweed pollen air-indexes have been recorded and the areas where the heaviest concentrations of air-borne pollen have been found. In British Columbia, the only 2 stations available had indexes below 1. Recent ground surveys have also indicated that little ragweed is present in any part of this province. Kennedy (13) found that ragweed pollen in Alberta was not very abundant in the air except at Medicine Hat where the average air-index was still below 10. Ground surveys by Moss (16) showed that common or short ragweed *Ambrosia artemisiifolia* var. *elatior* was prevalent around Medicine Hat and in a narrow strip east and west of Empress. In Saskatchewan no stations had ragweed pollen air-indexes above 2, although information is still not available from the extreme southern portion of the province. From ground surveys conducted in the summer of 1958 it is apparent that the *Ambrosia* relative, marsh elder *Iva xanthifolia*, is abundant in the southern portion of Saskatchewan. Since allergists believe pollen of marsh elder is quite as toxic as that of ragweeds, it may be that ragweed indexes for southern Saskatchewan will be found to be higher than for the rest of the province. Results from 8 stations established in Manitoba by Walton and Dudley

TABLE 1—DATA ON AIR-BORNE RAGWEED POLLEN FOR 44 ONTARIO STATIONS FROM 1950 TO 1958

Locality	Fig. ref. no.	Period of record	Pollen grains per cu. yd. of air			Average		Lowest annual index	Highest annual index	No. of years with index above 10. (Years of record in brackets)
			Average no. of days per year above 25 per day	All-time maximum (daily)	Average annual total count	Continued period of peak ragweed pollen count	Ragweed pollen air-index			
Cornwall	1	1953–54	15	180	1170	Aug. 21–Sept. 6	22.2	15.1	29.2	2 (2)
Ottawa	2	1950–58	11	173	809	Aug. 25–Sept. 8	15.1	6.1	22.7	6 (9)
Smiths Falls	3	1957–58	7	187	688	Aug. 24–Sept. 6	11.7	4.0	19.3	1 (2)
Mallorytown	4	1950–52	20	364	2055	Aug. 23–Sept. 15	33.2	23.5	41.0	3 (3)
Westport	5	1957–58	3	97	304	Sept. 1–Sept. 3	4.9	4.5	5.2	0 (2)
Picton	6	1956	24	258	2311	Aug. 18–Sept. 13	38.2	—	—	1 (1)
Belleville	7	1956	19	209	1822	Aug. 21–Sept. 13	30.2	—	—	1 (1)
Madoc	8	1957–58	10	252	722	Aug. 30–Sept. 11	15.4	12.7	18.0	2 (2)
Bancroft	9	1955–57	5	144	390	Aug. 29–Sept. 4	8.1	2.3	13.7	1 (3)
Barry's Bay	10	1957–58	1	58	105	Sept. 3	1.4	0.5	2.2	0 (2)
Chalk River	11	1954–56	2	194	277	Sept. 1–Sept. 4	4.5	1.6	9.8	0 (3)
Algonquin Park	12	1952–55	7	508	716	Aug. 29–Sept. 8	12.3	5.7	20.0	2 (4)
Haliburton	13	1956–58	1	50	118	Aug. 29–Aug. 30	1.9	0.6	3.5	0 (3)
Dorset	14	1952–54	4	86	277	Aug. 29–Sept. 2	6.1	2.0	8.8	0 (3)
Huntsville	15	1953–56	6	216	501	Aug. 29–Sept. 5	9.4	4.0	19.0	1 (4)
Rosseau	16	1957–58	2	47	146	Sept. 1–Sept. 2	2.7	2.0	3.3	0 (2)
Port Carling	17	1955–57	6	112	555	Aug. 27–Sept. 4	9.6	4.3	13.4	2 (3)
Muskoka Falls	18	1955–57	3	79	208	Aug. 30–Sept. 4	4.6	0.4	7.3	0 (3)
Beausoleil Island	19	1950–53	9	317	887	Aug. 25–Sept. 7	14.8	7.4	22.7	3 (4)
Gravenhurst	20	1955–57	11	144	940	Aug. 20–Sept. 13	16.8	4.0	23.3	2 (3)
Midland	21	1954	8	94	522	Aug. 23–Sept. 7	11.5	—	—	1 (1)
Peterborough	22	1953–54	19	342	2315	Aug. 18–Sept. 9	33.4	29.3	37.5	2 (2)
Toronto	23	1953–57	22	860	2942	Aug. 22–Sept. 19	44.1	24.7	55.3	5 (5)
London	24	1953–54	23	436	2569	Aug. 21–Sept. 17	38.5	31.5	45.5	2 (2)
Point Pelee National Park	25	1950–52	22	731	2609	Aug. 20–Sept. 17	38.5	28.9	49.7	3 (3)
Parry Sound	26	1955–56	12	284	1147	Aug. 21–Sept. 12	19.4	15.7	23.1	2 (2)
Magnetawan	27	1957–58	2	72	137	Sept. 2–Sept. 3	2.7	0.7	4.6	0 (2)
South River	28	1957–58	1	25	79	Sept. 3	1.2	0.6	1.8	0 (2)
North Bay	29	1951–53	5	148	370	Sept. 5–Sept. 9	7.5	3.8	11.1	1 (3)
Temagami	30	1954–55	2	43	90	Sept. 5–Sept. 6	2.4	1.7	3.0	0 (2)
New Liskeard	31	1956–57	0	11	29	—	0.3	0.3	0.3	0 (2)
Kapuskasing	32	1951–52	0	14	69	—	0.5	0.5	0.4	0 (2)
Sudbury	33	1954–55	2	83	171	Sept. 2–Sept. 3	3.4	1.6	5.1	0 (2)
Espanola	34	1956–58	3	36	258	Aug. 30–Sept. 1	4.2	3.4	4.9	0 (2)
Mindemoya	35	1952–55	5	97	384	Aug. 26–Sept. 2	7.7	4.5	10.6	1 (4)
Tobermory	36	1956–57	3	83	256	Aug. 31–Sept. 4	5.2	3.4	6.1	0 (3)
Blind River	37	1956–57	2	47	144	Aug. 31–Sept. 3	2.5	0.2	4.8	0 (2)
Sault Ste. Marie	38	1952–54	4	94	290	Aug. 27–Sept. 2	6.2	2.3	11.4	1 (3)
Black Sturgeon Lake	39	1952	1	40	173	Aug. 27	2.3	—	—	0 (1)
Port Arthur	40	1957–58	1	25	95	Aug. 22	1.2	0.2	2.1	0 (2)
10 m. S.W. of Fort William	41	1956	0	4	14	—	0.1	—	—	0 (1)
Fort Francis	42	1956–57	1	25	60	Aug. 22	1.0	0.3	1.7	0 (2)
Kenora	43	1956–58	4	167	297	Aug. 25–Aug. 27	6.2	1.6	10.3	1 (3)
Cedar Lake	44	1952–54	2	97	174	Aug. 23–Aug. 25	3.4	0.2	6.5	0 (3)

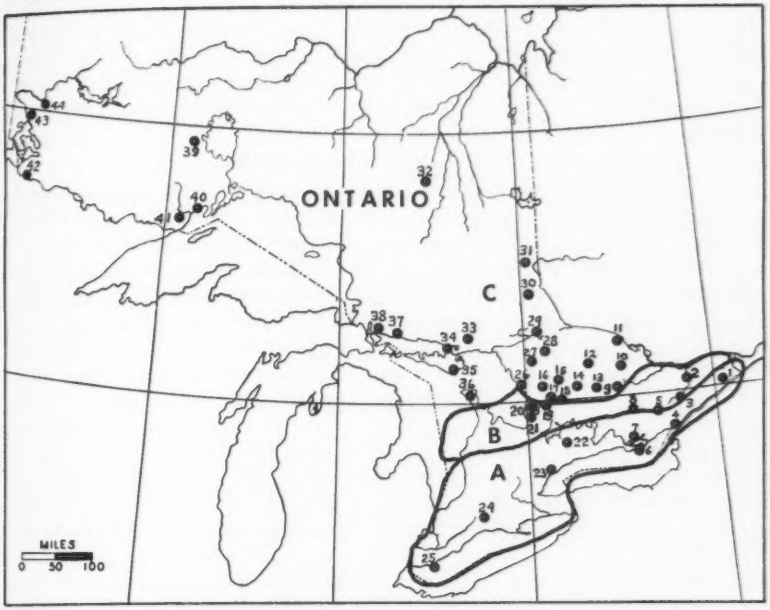

FIGURE 2. Zones of air-borne ragweed pollen intensity in Ontario. Numbers refer to the localities in Table I.

Zone A — average ragweed pollen air-indexes above 20.
Zone B — average ragweed pollen air-indexes between 10 and 20.
Zone C — average ragweed pollen air-indexes below 10.

(17) in 1939 indicated that ragweed indexes were generally low except in the area around Morden. Ground surveys conducted in 1957 by the author showed that common ragweed was well established at several locations, especially along roadside ditches and about communities between Winnipeg and Emerson along the Red River. Large infestations of the giant ragweed *Ambrosia trifida*, the perennial ragweed *A. psilostachya* var. *coronopifolia* and marsh elder were also recorded in this area.

The air-borne ragweed pollen results for 44 Ontario stations are summarized in Table I. "The average continued period of peak ragweed pollen count" was based on that period, considering all years of record, where not more than 4 days separated dates with counts of 25 or more pollen grains per cubic yard of air per day.

In Figure 2 an attempt has been made to indicate zones of air-borne ragweed pollen intensities for Ontario from the results of pollen collecting stations and ground surveys. In 1950, a paper by the author (2) based on herbarium specimens and ground survey information, showed distributional maps of the three species occurring in Eastern Canada, namely common, giant and perennial ragweeds. Of these 3 species, common ragweed appeared to be the most abundant and widespread, especially in southern Ontario and Quebec. Although giant ragweed is widely distributed, stands are very localized except in the extreme southern portion of Ontario. Only

occasionally has the perennial ragweed been found in Eastern Canada. Other related species such as marsh elder, false ragweed *Franseria* spp. and cocklebur *Xanthium* spp., although present in some parts of Eastern Canada, are not in sufficient abundance to produce significant amounts of pollen.

DISCUSSION AND CONCLUSIONS

Although the most northerly points where average ragweed pollen air-indexes exceed 10 are known to lie mainly in Eastern Canada it has not been clearly understood how far these limits extended. From Figure 1 it can be seen that a line from the base of the Bruce Peninsula to the St. Lawrence River about midway between the mouth of the Saguenay River and Quebec City is the northern limit of stations with indexes above 10. As also indicated, no pollen collecting stations in Western Canada have had indexes over 10 except for a small area around Morden, Manitoba.

In Manitoba, very little air-borne ragweed pollen information has been obtained since 1939. Walton and Dudley (18) in 1947 stated that common ragweed, although previously reported in Manitoba, had not been seen on any of their ground field surveys. As mentioned previously a survey conducted by the author in 1957 showed that common ragweed was widely scattered at several locations between Winnipeg and Emerson along the Red River. Giant ragweed, perennial ragweed and marsh elder were also common along this section. If new pollen-collecting stations were established between these places for a few years, some would probably show average ragweed indexes above 10.

Approximately two-thirds of the total area of heavy ragweed pollen-concentration in Canada lies in southern Ontario. In this paper the air-borne ragweed pollen data for 44 stations in Ontario have been reported. It would appear from these results that any site in zone A (Figure 2) would have a ragweed pollen air-index averaging above 20; in zone B, except for Westport, between 10 and 20; and in zone C, except for Algonquin Park, below 10. The average continued period of peak ragweed pollen counts for all stations in zone A is 24 days from August 21 to September 14; for stations in zone B it is 15 days from August 25 to September 9; and in zone C it is only 5 days from August 31 to September 4.

It is probable that the ragweeds and their air-borne pollen are increasing northward in Ontario. Several stations located in zone C (Figure 2) including Bancroft, Algonquin Park, Huntsville, Port Carling, North Bay, Mindemoya, Sault Ste. Marie and Kenora had at least one season out of three in which the ragweed index exceeded 10. Although it is possible that much of this pollen came from distances outside these areas, ground surveys have revealed that the common ragweed and occasionally giant ragweed are well established nearby at all these places except at Kenora. Giant ragweed has been previously reported for Kenora. In 1934-35 the average ragweed pollen air-index for Parry Sound was 8, whereas in the period 1955-56 the average index was over 19. This considerable increase over a 20-year period suggests that the ragweeds are now more abundant locally. Recent ground surveys have shown that all three species are widely scattered in and about Parry Sound and the common ragweed especially appears abundant enough to shed large amounts of pollen.

Unless the ragweeds, especially common ragweed, are effectively controlled, they will become more abundant in many of these northern localities and will spread still farther northward as new areas are opened. According to Moss (16) there is evidence that common ragweed can persist as far north as latitude 53°30′. In the Gaspé Peninsula and the Atlantic Provinces ragweed control campaigns have been conducted for several years with very encouraging results. In New Brunswick the ragweed pollen air-indexes at several stations have been decreased as much as 75 per cent since the inception of ragweed control campaigns.

Now that the broad outlines of ragweed pollen incidence in Ontario and Quebec are better known it would be advantageous to intensify ragweed control campaigns in the more northerly areas where the indexes are under or not much over 10.

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CONTROL OF THE EUROPEAN WIREWORM *AGRIOTES OBSCURUS* (L.) IN NOVA SCOTIA WITH INSECTICIDES APPLIED TO THE SOIL[1]

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ABSTRACT

The wireworm *Agriotes obscurus* (L.) was controlled at Lunenburg, Nova Scotia, with insecticides incorporated into the soil. Aldrin or heptachlor at 4 lb. per acre, and lindane at 1.25 lb., each significantly reduced the number of larvae per square foot and increased the yield of dry matter of forage for 3 years after application. A single application of aldrin or heptachlor at 3 lb. per acre resulted in increased yields of oats during the second season after application, and of leguminous forage plants during the third, but potatoes grown immediately after the application were not significantly less injured by wireworms than those grown on untreated check plots.

INTRODUCTION

The European wireworm *Agriotes obscurus* (L.) is established near several ports in Nova Scotia (1), and also near Agassiz, British Columbia, where Wilkinson (3) tested several insecticides for its control. Land of the Canada Experimental Project Farm at Lunenburg, Nova Scotia, has been severely infested for at least 20 years and great damage has been caused to hay, cereal, root, and potato crops. This is a report on experiments conducted during 1953-1957 on the effectiveness of aldrin, heptachlor, and lindane against this insect when applied to the soil under conditions approximating normal farm practice. The crops grown on the plots were potatoes, oats, and hay.

MATERIALS AND METHODS

In the spring of each year, 0–20–20 fertilizer was applied to all the plots at 500 pounds per acre. The insecticides were broadcast as sprays or in a granular carrier and then harrowed into the soil. To evaluate the insecticides, soil samples 6 inches square and 8 inches deep were sifted through a screen of quarter-inch mesh; by this method nearly all the larvae over 4 mm. long were collected. Samples of green forage were taken by swathing a strip 28 inches by 25 feet across each plot with a Jari mower[2]. The samples were dried at 175° F. before weighing. The oats were swathed similarly and threshed.

In the experiment where only a forage crop was grown, three treatments were replicated three times and randomized in plots 33 feet square, each with an adjacent untreated plot of equal size in a split-plot design. The three insecticides were: 20 per cent aldrin emulsion, 20 per cent heptachlor emulsion, each at 4 pounds of toxicant per acre, and 25 per cent wettable lindane powder at 1.25 pounds; each was applied as a spray at 100 gallons of water per acre on July 30, 1953. The plots were then sown to a

[1] Contribution No. 1005, Research Station, Kentville, Nova Scotia.
[2] Jari Products Inc., Minneapolis, Minn.

TABLE 1.—MEAN NUMBERS OF LARVAE PER SQUARE FOOT AND MEAN YIELDS OF DRY MATTER FROM FORAGE AFTER APPLICATION OF INSECTICIDES TO SOIL ON JULY 30, 1953, AGAINST *A. obscurus*

Treatment	Toxicant lb. per acre	Larvae per sq. ft. (3-yr. mean)	Dry matter lb. per acre (3-yr. mean)
Aldrin[1] emulsion, 20%	4.0	0.7	3048
Untreated check	—	11.1	2396
Heptachlor[2] emulsion, 20%	4.0	0.3	2897
Untreated check	—	9.8	2015
Lindane[3] wettable powder, 25%	1.25	1.3	2640
Untreated check	—	9.6	1977
Difference necessary for significance at 5% level		5.0	325
Difference necessary for significance at 1% level		6.8	442

[1] 1,2,3,4,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5, 8-dimethanonaphthalene. (Shell Oil Co. of Canada Ltd., Toronto, Ont.).
[2] 1 (or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene. (Velsicol Corp., Chicago, Ill.)
[3] Gamma isomer of 1:2:3:4:5:6 hexachlorocyclohexane. (Green Cross Products, Ltd., Montreal, Que.).

hay mixture containing timothy and red and alsike clovers. The yield of leguminous forage plants was recorded in 1956. To sample the wireworm population, eleven samples of soil were taken at random in 1954 from each plot, and five each year in 1955 and 1957.

In the experiment with a rotation of potatoes, oats, and hay, four treatments, including an untreated check, were replicated three times at random in plots 15 feet by 100 feet. The insecticides were: 20 per cent aldrin emulsion, 5 per cent granular aldrin powder, and 20 per cent heptachlor emulsion; each was applied at 3 pounds of toxicant per acre on May 17, 1954. The granular formulation was applied with a Gandy fertilizer spreader[1]; the other treatments were applied as sprays at 100 gallons of water per acre. The potatoes were examined for wireworm injury in 1954; the yield of oats was recorded in 1955, and that of hay in 1956 and 1957. The wireworm population was not sampled until 1957. Results were tested for significance by an analysis of variance.

RESULTS AND DISCUSSION

Aldrin and heptachlor at 4 pounds and lindane at 1.25 pounds per acre each gave marked reduction in the number of wireworms for 4 years after application (Table 1). The yields of dry matter from forage increased for 3 years after application. These increases were followed in 1957 by irregular declines to near the yields in the untreated plots.

In the plots on which potatoes, oats, and hay were rotated, injury to potatoes was not reduced significantly by applying aldrin or heptachlor at 3 pounds per acre (Table 2). In 1955, the yield of oats was increased significantly by each treatment. The increase in yield of dry matter from green forage was greater in 1956 than in 1957 but in neither year was this significant. In 1956 all treated plots yielded significantly more leguminous

[1] E. S. Gandrud Co. Inc., Owatonna, Minn.

TABLE 2.—PERCENTAGES OF POTATOES INJURED AND YIELDS OF OATS, DRY MATTER OF GRASSES AND LEGUMES FROM FORAGE IN CERTAIN YEARS AFTER APPLICATION OF INSECTICIDES TO SOIL AT 3 LB. OF TOXICANT PER ACRE ON MAY 17, 1954, AGAINST *A. obscurus*

	1954	1955	1956		1957
	Potatoes injured %	Oats, bu. per acre	Grasses and legumes, cwt. per acre	Legumes only, cwt. per acre	Grasses and legumes, cwt. per acre
Aldrin emulsion, 20%	90.8	60.7	39.2	24.4	33.5
Aldrin granular, 5%	65.4	69.2	42.2	21.9	34.3
Heptachlor emulsion, 20%	70.4	66.0	44.2	26.3	34.3
Untreated check	100.0	42.2	34.8	17.7	31.1
Difference necessary for significance at 5% level	—	8.7	—	4.0	—
1% level	—	13.2	—	6.1	—

forage than the check plots. In 1957 no larvae were found in the treated plots and an average of 1.5 per square foot were found in the check plots; the low numbers in the check plots were attributed to cultivation in the previous years.

Evidently in fields where *A. obscurus* is numerous, spring applications of aldrin or heptachlor at the moderate rate of 3 pounds per acre do not adequately protect potatoes planted the same year. This was not unexpected since aldrin and heptachlor require about a year to approach full effectiveness against wireworms (2) and potatoes are particularly susceptible to these insects. To protect potatoes from injury of this kind other work by the senior author* has indicated that the insecticide should be applied to the soil in the late summer or fall before planting and when practicable this procedure is recommended in Nova Scotia. Oats sown a year after treatment were apparently well protected.

ACKNOWLEDGEMENT

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INCREASE IN YIELD AND PROTEIN CONTENT OF NATIVE BLUEBUNCH WHEATGRASS FROM NITROGEN FERTILIZATION[1]

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ABSTRACT

A native, bluebunch wheatgrass stand on a Brown soil in the South Okanagan district of British Columbia with a mean precipitation of 11 inches has shown striking response to nitrogen fertilization. With applications of ammonium nitrate broadcast annually at rates of 0 to 60 lb. of actual nitrogen per acre, yield was increased from 640 to 1060 lb. of dry matter in 1957 and from 678 to 1725 lb. in 1958. Protein content increased from 3.9 to 6.2 per cent and protein production per acre from 27 to 107 lb. Ground cover of *Agropyron spicatum* increased from 4.6 to 6.8 per cent, of *Poa secunda* from 2.0 to 2.7 per cent. The number of culms of *Agropyron spicatum* per hundred-point sample increased from 9.8 to 188.2.

INTRODUCTION

The major problem of grassland ranges in British Columbia is poor condition and consequent reduction in carrying capacity (6). Marked changes in plant cover and considerable soil erosion have occurred (5). Bluebunch wheatgrass has decreased in abundance and vigour and sagebrush has increased (5). This decline in condition of the range is particularly serious because the grasslands are limited in extent in relation to the timber range and are, therefore, of vital importance for spring, fall and winter grazing (6).

Restoration of the grasslands is a major management problem (6). Recently, increased yields of other native grasslands from application of nitrogen fertilizers have been demonstrated. On native western wheatgrass (*Agropyron smithii*) in North Dakota an increase in forage yield (12 per cent moisture) from 748 pounds to 2271 pounds per acre was obtained from 90 pounds of nitrogen (4). On Kentucky bluegrass (*Poa pratensis*) in Kansas an increase of protein production from 178 to 412 pounds per acre was obtained from 100 pounds of nitrogen (3). However, both of these associations occur at precipitation levels around 18 inches. It can be questioned whether nitrogen would be effective at lower precipitation levels. This paper reports the effect of fertilization on a bluebunch wheatgrass (*Agropyron spicatum*) association at 11 inches of precipitation.

MATERIALS AND METHODS

The site of the experiment was on a terrace near the southern end of Okanagan Lake, British Columbia. The soil is Penticton silt loam, a Brown soil, and the natural cover is an association of the native grasses *Agropyron spicatum* (bluebunch wheatgrass) and *Poa secunda* (Sandberg bluegrass), and *Artemisia tridentata* (sagebrush). The sagebrush was cut down to ground level with a heavy-duty rotary mower and left on the ground. Precipitation at the Summerland Experimental Farm weather station, located about 1 mile from the plots, has averaged 10.79 inches over the last 42 years and is fairly evenly distributed from month to month.

[1] Contribution No. 3, Research Station, Canada Department of Agriculture, Summerland, B.C.
[2] Research Officers, Plant Nutrition and Animal Science, respectively.

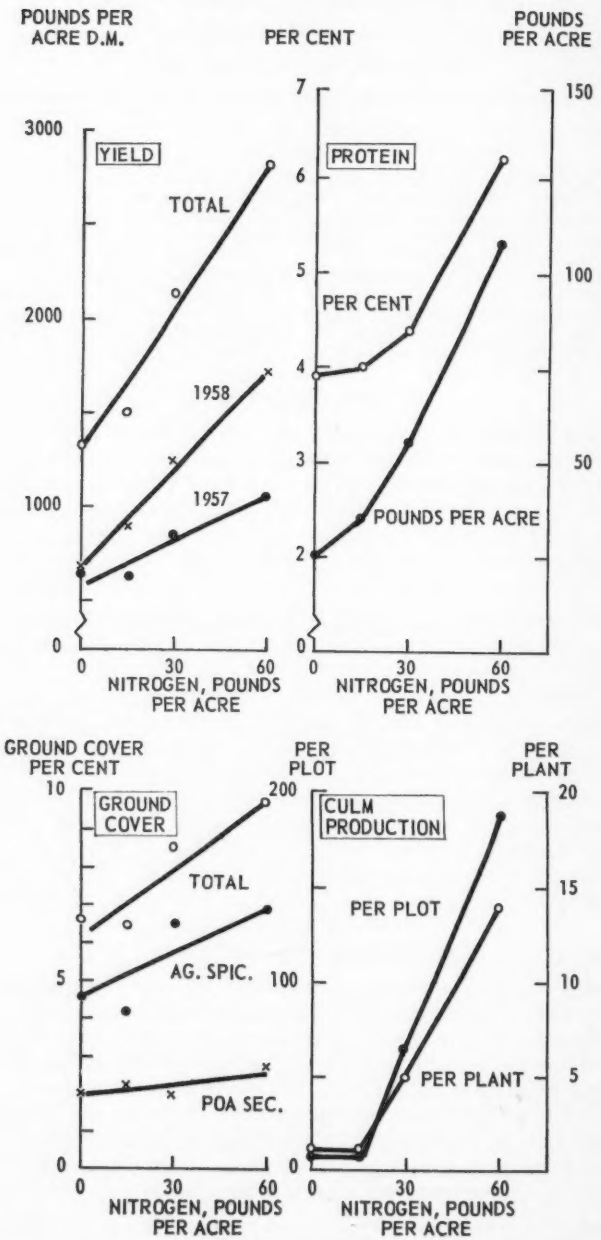

FIGURE 1. Effect of increasing levels of nitrogen fertilization of a native bluebunch wheatgrass stand on yield, protein content, density of ground cover and number of seedheads.

The experiment was located in an area which has not been grazed for several years and was not grazed during the experiment. The harvested material, therefore, included some forage standing from growth of previous years.

The experiment was designed as a 4-replicate randomized block test with four levels of nitrogen 0, 15, 30 and 60 pounds per acre—applied as ammonium nitrate. Plots were 10 feet by 100 feet. The fertilizer was applied broadcast in the spring of 1957 and again in the late fall.

In 1957, on June 14, two areas each containing 9.6 square feet and selected at random were harvested leaving 3-inch stubble. In 1958, on July 23, a strip 38 inches wide by 8 feet long was cut across each plot again leaving a 3-inch stubble. The yields are reported on a dry matter basis.

Crude protein was calculated as N x 6.25. Nitrogen was determined by the A.O.A.C. micro-Kjeldahl method (1).

A point sampling study of the population of grasses was made on the unharvested area of the experiment on October 15, 1958, by the method of Clarke *et al.* (2). Two 100-point samples were taken in each plot. In *Agropyron spicatum*, the dominant species, counts of the number of culms per sample and the average number per plant were also made.

RESULTS

Yield was sharply increased by applications of nitrogen as shown in Figure 1 and the increases were highly significant as shown in Table 1. In 1957, the zero nitrogen treatment yielded 640 pounds per acre and the 60 pounds of nitrogen treatment yielded 1060 pounds per acre, an increase of 65 per cent. In 1958, the zero nitrogen treatment yielded 678 pounds per acre and the 60-pound treatment 1725 pounds, an increase of 154 per cent. The average yield increase for 1957 and 1958 was 120 per cent.

Protein content of the forage was also sharply increased. It rose from 3.9 per cent in the zero nitrogen treatment to 6.2 per cent in the 60 pound treatment, an increase of 59 per cent. The increase in protein was even

TABLE 1.—TESTS OF SIGNIFICANCE FOR YIELD, PROTEIN CONTENT AND GROUND COVER OF A BLUEBUNCH WHEATGRASS ASSOCIATION FERTILIZED WITH NITROGEN

Factor	F value found	L.S.D. P = .05
Yield of forage, total 1957 and 1958, pounds per acre	35.7**	358
Yield of forage, 1957, pounds per acre	9.99**	206
Yield of forage, 1958, pounds per acre	10.8**	450
Protein, per cent	19.4**	0.81
Protein, pounds per acre	75.4**	13.4
Total ground cover, per cent	8.56**	1.59
Agropyron spicatum ground cover, per cent	4.86**	2.63
Poa secunda ground cover, per cent	N.S.	N.S.
Ag. spic. culms per plot	14.23**	71.8
Ag. spic. culms per plant	16.94**	4.84

*Significant at the P = .05 level
**Significant at the P = .01 level

more striking when expressed as pounds of protein per acre. Twenty-six pounds of protein per acre was obtained from the zero nitrogen treatment and one hundred and seven pounds of protein per acre from the 60-pound treatment, an increase of 312 per cent.

Ground cover was increased from 6.6 to 9.4 per cent when nitrogen was applied at 60 pounds per acre. This change was due mostly to *Agropyron spicatum*, which rose from 4.6 to 6.8 per cent, a 48 per cent increase. The other species present, *Poa secunda*, rose only from 2.0 to 2.7 per cent, a 35 per cent increase.

DISCUSSION

It has previously been shown that yield increases from nitrogen fertilization have been obtained from native western wheatgrass (4) and Kentucky bluegrass (3). In the work reported here, it is shown that nitrogen increased yield 120 per cent on a native bluebunch wheatgrass stand.

The western wheatgrass and Kentucky bluegrass associations are usually found on Black soils at precipitation levels around 18 inches. The bluebunch wheatgrass association on which this work was carried out occurs at much lower precipitation levels around 11 inches. It appears, therefore, that severe nitrogen deficiency of native range grasses may occur at widely-differing precipitation levels.

The work reported in this paper shows that nitrogen fertilization should be a useful practice for correction of poor range condition. The increase in yield of 120 per cent should result in a larger residue left on the range after grazing and an improvement in soil protection. An increase in ground cover from 6.6 to 9.4 per cent has been achieved after only 2 years of nitrogen application. In addition, the selective action of nitrogen in its effect on *Agropyron spicatum* over *Poa secunda* is noteworthy and suggests that an upgrading of the distribution of species will take place. The tremendous increase in culm production with its potential increase in seed production also suggests a long-term increase in the population of *Agropyron spicatum*.

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ALSIKE CLOVER POLLINATION BY HONEY BEES IN THE PEACE RIVER REGION[1]

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ABSTRACT

Pollination studies involving zero, one and three colonies of honey bees per acre, on commercial alsike clover fields ranging in size from 15 to 75 acres, were conducted in 1954, 1955, and 1957 in the Hinton Trail district of the Peace River Region of Alberta. These studies indicated that in large fields one colony of honey bees per acre is sufficient to pollinate alsike clover, consideration being given to competition of other crops and to the climatic conditions. The influence of competing crops was such that 65- to 75-acre fields, with one colony per acre, were similar in honey bee populations and seed production to 15- to 20-acre fields with three colonies per acre. Competing plant species accounted for 15 to 36 per cent of the pollen collected by honey bees. Fields with the higher populations of pollinators matured earlier than fields deficient in pollinators. A population of 3400 honey bees per acre (0.7 bee per sq. yd.) throughout the flowering period resulted in seed sets up to 82 per cent and seed yields to 375 lb. per acre. Check fields, where no honey bees were supplied, ranged in production from 29 lb. per acre, with a low native pollinator count, to 293 lb. where a pollinator equivalent of 1300 bumble bees per acre was observed. Bumble bees worked alsike clover at the rate of 28.6 florets per minute, as compared to 20.0 for leaf-cutter bees and 18.7 for honey bees.

INTRODUCTION

Alsike clover, *Trifolium hybridum* L., is an important legume seed crop in the northern sections of Alberta and British Columbia, which contribute approximately 90 per cent of the 5,900,000 pounds produced annually in Canada (4). As alsike clover is highly self-sterile (1, 2, 7) a pollinating agent is required for a high seed set.

Honey bees are excellent pollinators of alsike clover (3, 5, 6, 9). However, the populations required for effective and economical pollination are yet to be resolved. The effect of competing crops and the part played by native bees are factors worthy of consideration.

Field-scale studies were conducted in 1954, 1955 and 1957. Severe winter-killing of alsike clover precluded studies in 1956.

MATERIALS AND METHODS

The area of experimentation was located on a heavy-textured Gray Wooded soil in the Hinton Trail district of the Peace River Region of Alberta, the geographical position being Latitude 55°04′ North and Longitude 119°33′ West. Alsike clover, sweet clover and red clover are grown extensively for seed in this district.

Three alsike clover fields ranging from 15 to 75 acres were selected about mid-June each year, care being taken to have stands not older than three years. The test fields had been under cultivation for 10 years or

[1]Contribution from the Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.
[2]Apiculturist and Agronomist, Forage Crops, respectively.

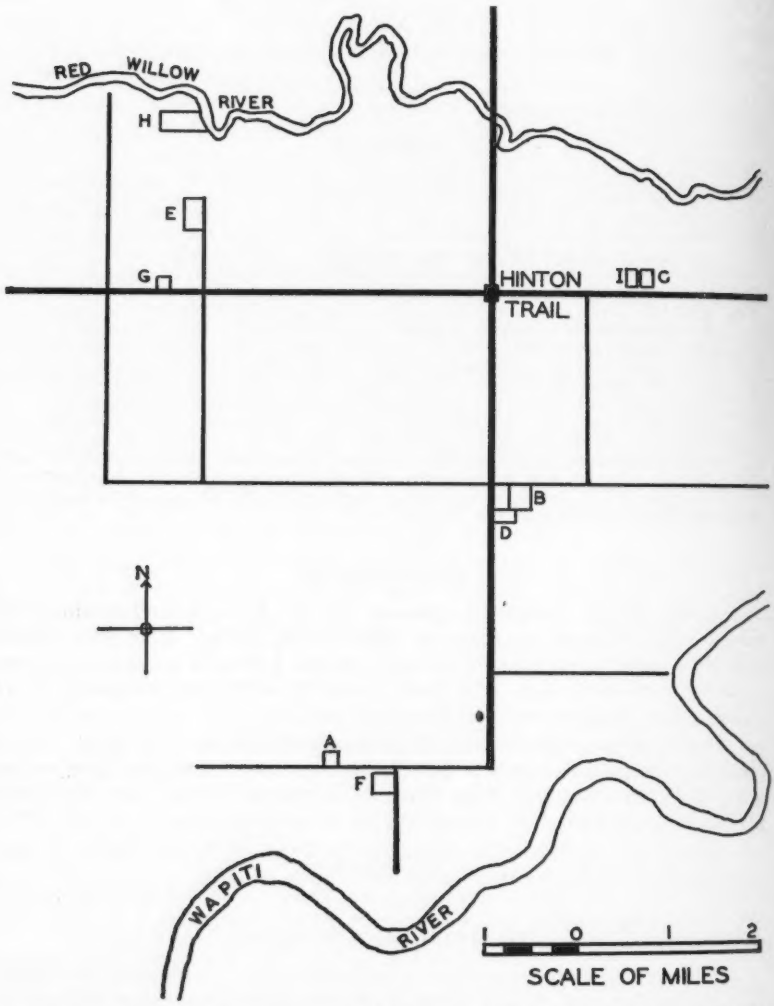

Figure 1. Location of the experimental alsike clover fields in the Hinton Trail District.

more, with the exception of one field (Field F) which had been cleared and broken 4 years earlier. An isolation distance of at least 1½ miles between test fields was provided. As it was not possible to obtain complete isolation from competing crops, the problem of competition for pollinators prevailed throughout the years of experimentation. The locations of the fields, A,B,C, (1954); D,E,F, (1955); G,H,I, (1957), are shown in Figure 1.

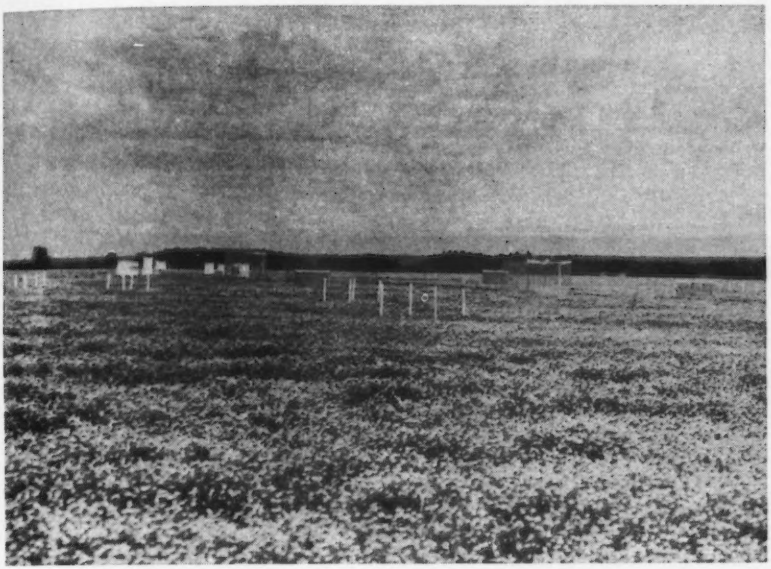

Figure 2. Part of Field H with colonies, cages and counting stations.

Honey bees were placed in the fields at concentrations of three and one colonies per acre at the beginning of alsike clover bloom. One field without bees was used as a check. Colonies were weighed immediately after placement and on completion of pollination to determine honey production during this interval.

Prior to placement of bees, three 4 × 4 × 4-foot lumite saran screen cages were placed in each field to exclude pollinators. In 1955 one 6 × 6 × 6-foot cage with a 5-frame nucleus of honey bees was located on each of Fields D and E. In 1957, two 6 × 6 × 6-foot cages with honey bee nuclei were distributed on each of Fields G, H, and I.

Counting stations (10 in 1954 and 1955 and 15 in 1957) of 2 square yards in area were selected at random in each field. Counts of honey bees and native bees were taken for 1-minute periods for each station during several days throughout the period of bloom to determine pollinator populations. These counts were discontinued on all fields when the bloom on any one field had decreased to 5 per cent of full bloom as determined by visual estimates. Figure 2 shows part of Field H with colonies, cages and counting stations.

Visitation rates of honey bees and native bees were determined by recording the number of florets visited by each bee during periods of not less than one minute.

Insect sweeps were made during maximum bloom with a 12-inch net. In 1954 and 1955, the injurious insect complex of lygus bug, aphid and leaf hopper species was less than one per sweep. In 1957, the count was 2.5 per sweep, with the major portion of the complex consisting of lygus

TABLE 1.—EFFECTS OF VARYING CONCENTRATIONS OF HONEY BEES ON ALSIKE CLOVER SEED PRODUCTION

	1954				1955					1957				
	Field			Caged	Field			Caged		Field			Caged	
	A	B	C	No bees	D	E	F	Bees	No bees	G	H	I	Bees	No bees
Size of alsike fields in acres	15	75	30		20	65	30			15	65	30		
Age of stand in years	2	2	3		3	2	2			2	2	2		
Concentration of honey bees in colonies per acre	3	1	0		3	1	0			3	1	0		
Pollinator populations per square yard:														
Honey bees	0.60	0.60	0		0.58	0.34	0.05[1]			0.68	0.67	0.05[1]		
Native bees	0.20	0.05	0.10		0.10	0	0.34			0.01	0.02	0.04		
Per cent bloom on last counting date[2]	10	5	20		5	10	25			20	5	40		
Analysis of pollen collected from pollen traps with plant sources in per cent—														
Alsike clover	85	77			75	64				76	72			
Sweet clover	10	11			13	20				23	26			
Others[5]	5	12			12	16				1	2			
Analysis of alsike clover head samples														
Florets per head	63	67	61	62	61	65	66	59	62	70	69	63	70	60
Per cent pods formed	77	82	18	4	75	65	47	77	1	62	74	14	81	10
Seeds per pod	2.2	2.2	2.1	2.2	2.0	2.0	2.3	1.8	1.7	2.4	2.4	2.5	2.4	2.4
Honey production in pounds per colony[3]	109[4]	128			165	155				196	188			
Alsike seed yields in pounds per acre	251	370	29	13	246	210[6]	293	275	25	194[6]	375	60	360	40

[1] Honey bees from apiary of 5 to 6 colonies ½ to ¼ mile distant.
[2] Last pollinator counting date was August 6 in 1954 and 1955 and August 7 in 1957.
[3] L.S.D. at 5% level was 15, 26 and 26 lb. for 1954, 1955 and 1957 respectively.
[4] Some of the colonies had European foulbrood which was controlled with antibiotics.
[5] Includes red clover, fireweed, etc.
[6] Partial to heavy lodging.

bug (predominantly *Lygus oblineatus* Say.) and aphid species in equal proportions. No insecticides were applied to the fields although they may have been warranted in 1957.

Pollen from traps attached to five colonies of average strength on each field was used to determine pollen preference of honey bees. The pollen, collected approximately every third flying day, was air-dried and weighed. A representative sample of 200 pellets from each date was analysed for plant source on the basis of colour and microscopic examination.

Prior to harvesting, random samples of 10 heads were selected from each counting station and caged area. These heads were analysed for number of florets, per cent of florets with seed and number of seeds per pod. In addition to the caged areas, twenty 2-square-yard areas from each field including the counting stations were harvested for seed yield. Table 1 contains a description of the nine fields used for this study and a summary of the data obtained each year.

RESULTS AND DISCUSSION

The average pollinator populations for each season show that in 1954 and 1957 the honey bee populations in the experimental fields with bees were identical for each year despite the heavier concentrations of colonies placed in Fields A and G. In 1955 the population of Field E was lower than Field D partly because of the greater proximal acreage of competing crops as shown by the pollen analyses. The variability in native bee populations between fields and between years is evident. Bumble bees comprised over 95 per cent of the total wild bee population observed. The native bee species observed were identified as follows:

Bombidae
- *Bombus terricola* Kby.
- *B. sylvicola* Kby.
- *B. vagans* Sm.
- *B. ternarius* Say
- *B. nevadensis* Cress
- *B. auricomus* (Robt.)
- *B. rufocinctus albertensis* Ckll.
- *B. rufocinctus prunellae* Ckll. and Porter
- *B. rufocinctus rufocinctus* Cress

Megachilidae
- *Megachile frigidus* Sm.
- *M. melanophaea wootoni* Ckll.

The average rate of visitation of honey bees and the several species of bumble bees was calculated at 18.7 and 28.6 florets per minute, respectively, indicating that bumble bees are individually one and a half times as effective for pollination of alsike clover as honey bees. Although leaf cutter bees were present in negligible numbers in the 3 years of experimentation, they were observed for visitation rates in 1954 only and this rate, based on three different observations, was 20 florets per minute.

The pollen analyses indicate the part played by competing plants, such as sweet clover, red clover and fireweed, in attracting varying percentages of pollen-carrying field bees. Neighbouring alsike clover fields undoubtedly contributed a part of the total alsike clover pollen.

Evidence for the need of adequate pollination for high seed yields is reflected in the analysis of alsike clover heads. The variation in florets per head may be attributed to differences in stands. There was no significant variation in seeds per pod with the exception of Field F. The seed set, as judged by the per cent of florets forming pods, was closely correlated to the seed yields with the exception of Field F where the superior stand counterbalanced the lower seed set. Although analysis of individual heads showed that a 96 per cent seed set is possible, the highest set for any one field was only 82 per cent. This difference is attributed to climatic factors which inhibited bee activity although consideration was given the reason advanced by Pedersen *et al.* (8) that the ability of plants to develop pods from cross-pollination is limited. In all 3 years the average maximum temperatures for July, when most of the pollination was effected, varied from 1.1 to 5.2 degrees below the normal of 72.1 degrees F., while the combined rainfall for July and August in each year varied from 0.32 to 3.87 inches above the normal of 4.47 inches. Other than July, 1954, and August, 1955, the hours of sunshine were below normal. These conditions, besides hindering bee activity, were conducive to lodging of the alsike clover.

Fields with the higher seed set also ripened earlier as shown by the lower per cent bloom on the last counting date. Adequate pollinator populations would result in hastening maturity, thus escaping damage from early frosts prevalent in this region.

Honey production in some of the colonies in Field A was reduced because of European foulbrood infection. In 1955 and 1957 the honey production per colony for the different concentrations did not differ significantly. The colony yields indicate that the foraging area of the honey bees was not confined to the experimental fields.

Seed yield data were analysed by the "t" test analysis of difference. Field B yielded significantly more than Field A. Field E was significantly lower than Field F. Fields G, H and I were highly significantly different from each other. The seed yield of Fields E and G was reduced by lodging. Even though, in 1957, some of the cages excluding bees were damaged, allowing some pollinators to enter, the exceedingly high variation in yields of the caged areas with and without pollinators indicate the importance of cross-pollination in alsike clover seed production. The yield of Field F was partly attributed to the excellent stand. An important feature of Fields A and F was their subjection to comparatively higher native bee populations, since they were on the edge of a farming area.

The populations of honey bees and native bees, and their respective rates of visitation, would suggest that a population of 3400 honey bees per acre (0.7 honey bee per square yard) is satisfactory for an adequate alsike clover seed set. This figure corresponds to the 3000 honey bees per acre proposed by Dunham (5) and the range of one-third to one bee per square yard for yields of 175 to 800 pounds per acre observed by Tucker *et al.* (9).

The estimated acreage of competing crops within bee-flight range of the experimental fields with one colony per acre, was equal to or greater than the experimental fields. One colony of honey bees, therefore, may

suffice for 2 acres of alsike clover in a large scale pollination program with competing crops at a minimum. As such conditions rarely occur in this region, a recommendation of one colony per acre for fields in excess of 50 acres is justified.

CONCLUSIONS

One colony of honey bees per acre on alsike clover fields in excess of 50 acres is required for satisfactory pollination. On small acreages and with competing crops in close proximity, greater concentrations of up to three colonies per acre may be necessary. An equivalent population of 3400 honey bees per acre resulted in seed sets up to 82 per cent and seed yields to 375 pounds per acre under the conditions of the stands and the climatic factors prevailing. Individually, bumble bees are 1.5 times as effective as honey bees but their variable populations make them unreliable for commercial alsike clover seed production.

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NOTE ON AN ELECTROLYTIC PROCEDURE FOR DIFFERENTIATING BETWEEN FROST INJURY OF ROOTS AND SHOOTS IN WOODY PLANTS[1]

Frost injury to woody plants may be the result of injury to the root system, the shoot, or both. In field tests such injury is generally measured by the growth of the plant after exposure. Such an index does not differentiate between the two possible types of injury. Since it has been shown (1,2) that the electrolytic method is valuable in determining the hardiness of shoots, the present studies were conducted to determine the value of the procedure in the differentiation between injury to roots and to shoots.

Plants used in this study were grown outdoors in 8-inch metal containers and were hardened and reached autumn maturity under the same conditions until November 15, 1957. Two treatments were then applied: (a) outdoor exposure of the plants to low temperature conditions, during which the air temperature dropped to -12°F. and the soil temperature to 0°F.; (b) check treatment, in which the plants were stored at 32°F. ± 3°F. from November 15, 1957. During storage the plants were supplied with artificial illumination at an average intensity of 32.8 watts per square foot, regulated to conform with length of natural daylight.

Figure 1, taken on July 30, 1958, shows the differences in recovery after the treatment terminated on January 13, 1958, of 1-year-old apple varieties of: (1) Northern Spy; (2) McIntosh; (3) Antonovka, all grown on *M. robusta 5* rootstock. It is clearly shown that all the plants have survived the storage condition but not the outdoor exposure. These differences were related to the electrolytic readings (e.r.) of shoots and roots which were determined on January 13 and again on February 11, 1958, and are presented in Table 1. All determinations within each variety are shown as averages of two trees, excepting the outdoor treatment on February 11 which is based on one tree.

The readings of electrolytes diffused in water at room temperature of all outdoor twigs ranged from 175 to 256 micromhos (14 to 25 per cent of total) as compared with e.r. of twigs of check plants, which ranged from 121 to 187 micromhos (10 to 15 per cent of total). A previous study (2) showed that diffusion of electrolytes from such tissues within the above ranges was not associated with any injury to twigs when the roots were protected from freezing. It is, therefore, deduced that failure of growth of these plants was entirely due to root injury.

The data of Table 1 show that the average total electrolytes determined by diffusion in boiling water of roots of *M. robusta 5* was 1309 micromhos for check plants and 456 micromhos for the outdoor plants. In these tests, while the minimum air and soil temperatures, −12°F. and 0°F. respectively, occurred on January 3, the plants were subjected to a series of lethal root temperatures starting as early as December 13, 1957, when a minimum air and soil temperature of −6°F. and 1°F. respectively were recorded. Between the occurrence of soil temperatures of 0°F. and 1°F., which unpublished work (3) indicated as critical, the soil and roots thawed out due to soil temperatures ranging from 34 to 39°F. and the soil was

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FIGURE 1. Differences in recovery of 1. Northern Spy; 2. McIntosh; 3. Antonovka, all grown on *M. robusta 5* rootstock following *a*, root injury after outdoor exposure, and *b*, uninjured roots after winter storage. Tests have shown that the diffusion of electrolytes from injured roots was two to three times as great as the diffusion from uninjured roots.

TABLE 1.—ELECTROLYTIC READINGS OF WATER EXTRACTS FROM ROOTS OF *M. robusta 5* FOLLOWING SPECIFIED TREATMENTS

Date in 1958	Treatments	Shoot		Electrolytic readings expressed in Micromhos		Per cent
		Variety	Survival %	Total electrolytes extracted by boiling tissues in water	Diffused electrolytes from tissues into water at room temperature	Diffused electrolytes to total
Jan. 13	Outdoor exposure	N. Spy	0	355	300	85
		McIntosh	0	570	470	82
		Antonovka	0	465	335	72
		Mean	0	463	368	80
	Storage check	N. Spy	100	1325	415	31
		McIntosh	100	1400	465	33
		Antonovka	100	1205	400	33
		Mean	100	1310	427	32
Feb. 11	Outdoor exposure	N. Spy	0	450	300	67
		McIntosh	0	510	350	69
		Antonovka	0	390	310	79
		Mean	0	450	320	72
	Storage check	N. Spy	100	1230	335	28
		McIntosh	100	1290	420	32
		Antonovka	100	1405	370	27
		Mean	100	1308	375	29

frequently saturated. The marked differences in electrolytic readings suggest diffusion of electrolytes from injured root tissue during these warm periods. There is also the possibility of loss of electrolytes during the process of washing soil from roots while preparing these for the electrolytic test.

Unlike the treatment effects on total electrolytes extracted in boiling water, there was little or no effect on the amount of electrolytes diffused from the root tissue into water at room temperature. However, when the amount of electrolytes diffused at room temperature was expressed as per cent of the total extracted by boiling water, the e.r. of the water extract of outdoor roots averaged 76 per cent and the e.r. of check roots averaged 30 per cent.

These techniques, in conjunction with the technique for testing shoot injury (1,2) can be used to differentiate between the frost injury to roots and shoots of various overwintering plants. This method proved effective in determining both the total and partial injuries to shoots (2) and should be equally effective in studying various levels of root injuries.

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